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Influence of Oxygen Concentration on the Growth of Juvenile Largemouth Bass[1,2,3]

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Oregon State University, Corvallis, Oregon

ABSTRACT

Experiments are reported on the influence of nearly constant dissolved oxygen concentrations, both below and above the air-saturation level, and of wide diurnal fluctuations of oxygen concentration on the appetite, growth, and food conversion efficiency of juvenile largemouth bass, *Micropterus salmoides* (Lacépède). The experimental apparatus used was designed to provide constant flows of water at 26 C and with controlled oxygen content through 12-gal (45-liter) bottles each containing 10 test fish. The fish were fed unrestricted rations of small, live earthworms throughout the six experiments, whose duration was usually 15 days.

The growth rates and food consumption rates of the bass increased markedly with increase of the constant oxygen concentrations to levels near the air-saturation level, and declined with further increase of oxygen concentrations. Gross food conversion efficiencies were considerably reduced only at concentrations well below 4 mg/liter.

The growth of bass subjected alternately to low and higher oxygen concentrations for either equal or unequal portions of each 24-hr day was markedly impaired. It was almost always less than that which presumably would have occurred had the fish been held at a constant concentration equal to the mean, either arithmetic or geometric, of the higher and lower concentrations to which the fish had been exposed.

INTRODUCTION

Freshwater fishes may be exposed to widely varying concentrations of dissolved oxygen in their natural environments. In some waters, especially those receiving putrescible organic wastes such as municipal sewage, the dissolved oxygen may be reduced to low levels for long periods of time, or large diurnal fluctuations of the oxygen concentration may occur. Release of plant nutrients through oxidative decomposition of the organic matter by bacteria often results in increased growth of algae and other aquatic plants. Oxygen concentrations may rise to very high levels during the day because of photosynthetic activity of the green plants and may be greatly reduced at night because of continued plant and animal respiration and bacterial action on remaining putrescible organic matter. Tarzwell and Gaufin (1953) reported that at one station in Lytle Creek, a small stream in southern Ohio polluted with municiple sewage, the dissolved oxygen concentration was 19.4 mg/liter one afternoon and 0.7

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[2] Technical Paper No. 2175, Oregon Agricultural Experiment Station. A contribution from the Pacific Cooperative Water Pollution and Fisheries Research Laboratories, Oregon State University.

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mg/liter the next morning. Greater fluctuations of dissolved oxygen are possible in large, sluggish streams and in standing waters. The occurrence in ponds and lakes of oxygen concentrations approaching or exceeding 30 mg/liter has been reported (Wiebe, 1931; Woodbury, 1941).

Freshwater fishes can withstand wide variations of oxygen concentration. Doudoroff (1957) concluded that mortality of fishes at reduced oxygen concentrations near and above 3 mg/liter "is not to be expected ordinarily, even when the low concentrations persist for long periods." He further noted that much lower concentrations, near and below 1 mg/liter, may be tolerated not only by relatively resistant fishes but also by the more susceptible species under otherwise favorable conditions. Abnormally high oxygen concentrations have long ago been reported harmful and fatal to fish by Haempel (1928), but other investigators have reported that fishes could tolerate very high concentrations. Wiebe and McGavock (1932) found that various centrarchids, salmonids, and cyprinids withstood prolonged exposures to concentrations ranging from 20 to 34 mg/liter, or about two to more than three times the air-saturation levels, at temperatures of 14.5 to 17 C. Wiebe (1933) reported that fingerling largemouth bass, *Micropterus salmoides* (Lacépède), at approximately 17 C tolerated concentrations ranging from 29 to 40 mg/liter for 23 hr. It may not be assumed, of course, that fish surviving at very high or low oxygen concentrations are in good physiological condition and able to feed and grow at normal rates.

Results of experimental studies of the influence of dissolved oxygen on the growth, appetite, and food utilization efficiency of fishes have been recently reviewed and summarized by Doudoroff and Warren (1965). When the present investigation was undertaken, the inhibiting effects of subsaturation levels of dissolved oxygen on the growth of salmonid fishes (embryos, larvae, and juveniles) had received some attention. Little or no information was available, however, concerning the influence of concentrations above air-saturation levels and of wide diurnal fluctuations of oxygen concentration on the appetite and growth of fishes, and concerning the effects of persistent low concentrations on the growth of warmwater species. Doudoroff (1960) has pointed out that experimental data on the effects of the diurnal fluctuations of dissolved oxygen are needed as a factual basis for the establishment of sound criteria of water quality applicable to freshwater fish habitats where wide fluctuations occur. Bouck and Ball (1965) have demonstrated changes in the electrophoretic patterns of serum proteins in largemouth bass and in bluegills, *Lepomis macrochirus*, that had been subjected to a low oxygen concentration of 3 mg/liter for 8 hr of each of 9 successive days, but effects on growth were not reported.

Results are presented here of six laboratory experiments with juvenile largemouth bass. These experiments were performed during the period from April to November 1961, at temperatures near 26 C. In each experiment some groups of bass were exposed to various nearly constant oxygen concentrations near and below the air-saturation level; in four experiments, tests at constant concentrations above the air-saturation level also were included. In all but one of the experiments, additional groups of bass were subjected to wide diurnal fluctuations of dissolved oxygen. The growth and food consumption rates of the bass and their efficiencies of food conversion under the various experimental conditions were determined.

METHODS AND MATERIALS

The experimental apparatus used in this study was designed to maintain a constant flow of water with controlled temperature and dissolved oxygen content through seven vessels (large bottles) containing the test fish. The apparatus was located in a constant-temperature room illuminated with fluorescent lights. Figure 1 is a schematic drawing of the apparatus; only one of the seven test vessels is shown.

Filtered water supplied from a small spring-fed stream was introduced into an overflowing, 5-gal (19-liter), constant-head jar of Pyrex glass. From this jar the water was siphoned into a similar jar where it was heated to the desired temperature by a thermostatically controlled, stainless-steel immersion heater. The warmed water then flowed through a 1-gal (3.8-liter) jar containing the thermoregulator, and thence through a glass column where it was vigorously aerated. Next, the water flowed through a 1-gal (3.8-liter) distribution jar to seven overflowing vertical glass columns packed with Raschig rings. The desired level of dissolved oxygen in the water leaving each of these columns at the bottom was attained by introducing nitrogen or oxygen at an appropriate rate through a gas disperser near the lower end of the column.

After leaving a glass column, the water passed through a flow-adjustment stopcock and a 300-ml sample bottle into a stoppered, 12-gal (45-liter), Pyrex glass solution bottle, which served as the test vessel. A bypass tube connecting the inflow and outflow tubes of each test vessel made it possible to maintain a constant flow of water through the rest of the system when the stopper of a test vessel was removed. The water flowing out of each test vessel passed through a ball-displacement flowmeter, through another 300-ml sample bottle, and into a wooden reservoir, which received the effluents from all the test vessels. Part of the discharged water (70–80%) was then returned by a centrifugal pump to the jar containing the immersion heater, thus conserving heated water. A flow of about 500 ml/min was maintained through each test vessel.

The juvenile largemouth bass used in these experiments were seined from ponds in the Willamette Valley of Oregon. It was necessary to use fish from different sources; however, all fish used in any single experiment were from one source. The fish used in the first two experiments were from the 1960 brood; those used in the remaining experiments were from the 1961 brood. Mean total lengths of fish used in the different experiments ranged from 61.8 to 84.5 mm.

The test fish were held at the experimental temperature of 26 C for a period of 3 to 10 days in a 50-gal (189-liter) aquarium before use in an experiment. They were fed a mixed diet of earthworms, cladocera, and mosquito larvae until 24 hr before their transfer to the test vessels. Several fish of each

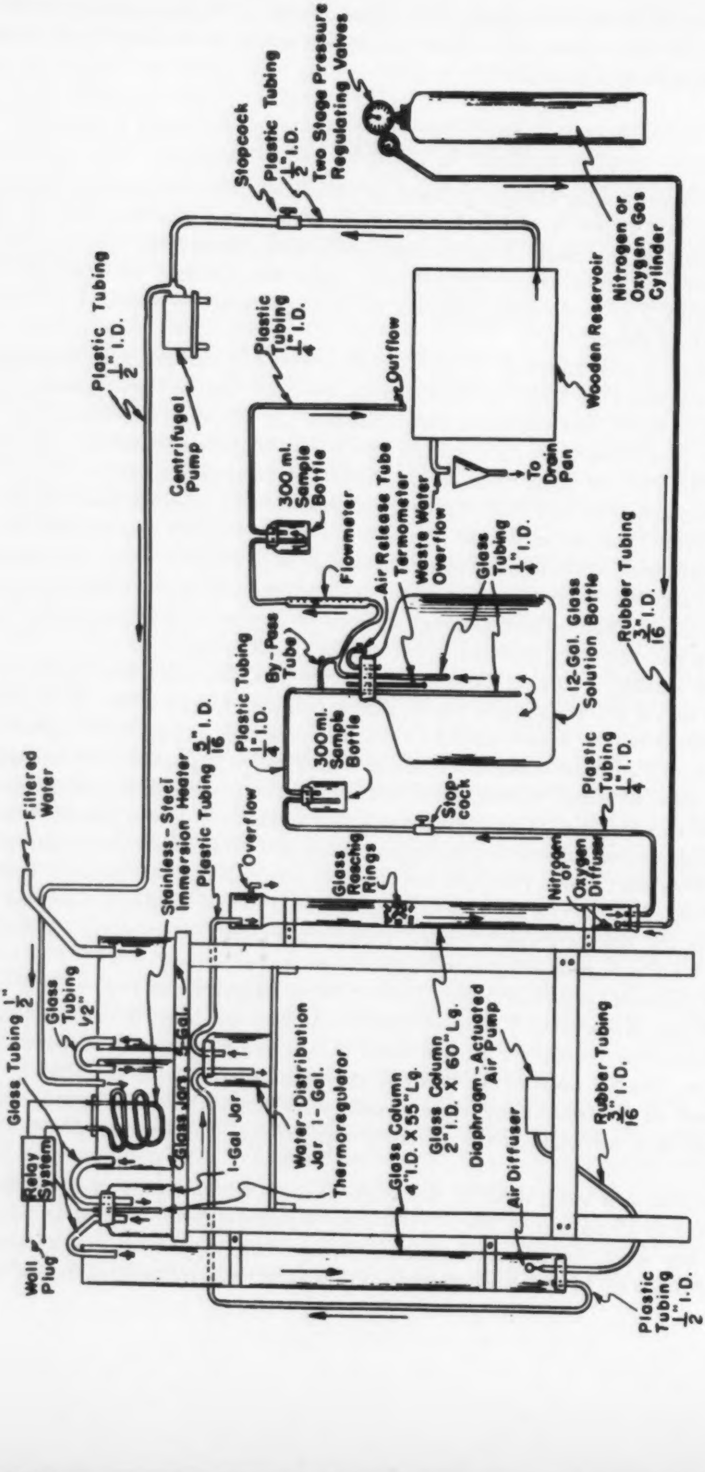
Wall Plug
Relay System
Glass Tubing 1/2"
Glass Tubing 1/2"
Filtered Water
Stainless-Steel Immersion Heater
Glass Jar, 5-Gal.
1-Gal Jar
Water-Distribution Jar 1-Gal.
Thermoregulator
Plastic Tubing 5/16" I.D.
Overflow
Glass Raschig Rings
Glass Column 4" I.D. X 55" Lg.
Glass Column 2" I.D. X 60" Lg.
Diaphragm-Actuated Air Pump
Air Diffuser
Rubber Tubing 3/16" I.D.
Plastic Tubing 1/2" I.D.
Nitrogen or Oxygen Diffuser
Plastic Tubing 1/4" I.D.
Stop-cock
300 ml. Sample Bottle
Plastic Tubing 1/4" I.D.
By-Pass Tube
12-Gal. Glass Solution Bottle
Rubber Tubing 3/16" I.D.
Air Release Tube
Termometer
Waste Water Overflow
Glass Tubing 1/4" I.D.
Flowmeter
300 ml. Sample Bottle
Centrifugal Pump
Plastic Tubing 1/2" I.D.
Plastic Tubing 1/4" I.D.
Outflow
To Drain Pan
Wooden Reservoir
Stopcock
Plastic Tubing 1/2" I.D.
Two Stage Pressure Regulating Valves
Nitrogen or Oxygen Gas Cylinder

FIG. 1. Schematic drawing of the experimental apparatus. Only one of seven test vessels is shown.

lot were examined for parasites and disease before the experiment. The fish used in experiment 5 had a mild bacterial infection which was successfully treated with aureomycin before the fish were placed in the test vessels.

For each experiment, fish of fairly uniform size were selected from the available stock, discarding the largest and smallest individuals. A group of 10 fish that had been weighed individually and measured under light anesthesia with MS 222 (tricaine methanesulphonate) was then placed into each test vessel. An extra sample of 10 fish was taken at the beginning of each experiment for determination of the ratio of dry weight to wet weight. This ratio was used in computing the initial dry weights of all the fish used in that experiment.

After the fish had been placed in the test vessels, the dissolved oxygen concentrations were adjusted to the desired levels over a period of about 8 hr. Experimental feeding of the fish was begun in the morning of the following day. Lights in the laboratory room were controlled by a timing device so as to come on at 6:00 AM and to go off at 10:00 PM, except during experiment 1, when the lights came on at 6:00 AM and went off at midnight.

The experimental conditions were maintained by making the necessary measurements and adjustments twice daily. The first check was made between 7:00 and 9:00 AM, and the second was made approximately 8 hr later. During these checks, water temperatures, dissolved oxygen concentrations, and water flows were determined. No adjustments of the apparatus were made until after the water samples had been taken. All dissolved oxygen determinations were made by the Alsterberg (azide) modification of the Winkler method.

In experiments 2 through 6, the fish in two or three of the test vessels were subjected to a low level and then a higher level of dissolved oxygen for definite portions of each day. In some of these experiments, the oxygen content of the water flowing into the test vessels was changed every 12 hr, being reduced at 10:00 PM and increased at 10:00 AM. In other experiments, the oxygen content of the inflowing water was reduced at either 4:00 PM or midnight and was increased always at 8:00 AM; the low level was thus maintained for either 8 or 16 hr each day and the high level for the remainder of the day.

In all experiments, the fish in each test vessel were fed small, live earthworms each day. In the first experiment, the fish were fed in the morning between 8:00 and 10:00 AM, and again at least 6 hr later; in all the other experiments, the fish were fed once a day between 8:00 and 10:00 AM. An attempt was made to supply enough earthworms at each feeding to ensure a surplus remaining at the time of the next feeding. The supply of food provided was exhausted by the time of the next feeding on a few occasions only, mostly in the first experiment. Uneaten earthworms and excrement were siphoned from the test vessels each time the fish were fed. All earthworms were weighed before being placed in the test vessels and all uneaten worms were weighed after removal from the test vessels in order to determine the quantity consumed. The earthworms were weighed out of water after excess moisture had been removed by blotting with paper towels.

In all experiments except the first (in which the earthworms were merely washed before use), the earthworms were held for approximately 20 hr in water at 26 C before they were fed to the fish. This was done to minimize changes in weight of the worms due to defecation or to osmotic uptake of water. However, the wet weights of four of five samples of worms taken at different times decreased by 3 to 15% after the worms had been held in water for 24 additional hr. The average loss in weight of these four samples was 7.5%. The first two samples of worms examined in July showed very little change in weight, the changes varying only from 1% gain to 3% loss. For this reason, the need for determination of changes in wet weight of the worms was not immediately realized, but subsequent tests showed that these changes could be considerable at other seasons of the year and should have been determined for each experiment. The amount of uneaten earthworms removed from the test vessels ranged from 7 to 40% of the amount fed, the average amount being 21%. A larger percentage of food was left uneaten in the test vessels having low oxygen concentrations than in test vessels having high concentrations. The wet weights of uneaten earthworms have not been corrected for the above-mentioned changes, because adequate data were not available for each experimental period and the weight changes of the five different samples of worms were highly variable. Thus, the values reported in this paper for total wet weights of food consumed by groups of fish are usually somewhat higher than the true values.

Dry weights of samples of the earthworms were obtained after drying until constant weight at 70 C. The "per cent dry weight" of earthworms (i.e. dry weight expressed as a percentage of the wet weight) changed while the worms were held in water. The per cent dry weight of worms that had been held in water for only 20 hr was higher than that of worms held in water for 24 additional hr. Also, the initial values for earthworms fed to the fish in different experiments were variable. Those for the worms that had been in water for 20 hr increased from about 16% in June to about 20% in late October. These seasonal variations in per cent dry weight of the worms were not clearly evident until experiment 6 was begun in October. Therefore, the per cent dry weight of the worms used as food was not determined for each experiment. A value of 17% was used to convert to dry weights the computed wet weights of food consumed in all experiments. This value is the mean per cent dry weight of six samples of worms taken at different times during the course of the experiments. Some of these samples had been held in water for 20 hr and some for 44 hr.

At the conclusion of an experiment, the concentration of oxygen was brought nearly to the saturation level in all test vessels. The fish were then held for 24 hr without food before their final weights were determined. After final wet weights had been obtained, all of the fish were dried at 70 C for at least 5 days and their dry weights determined.

RESULTS AND INTERPRETATION

Presented in Table I are the inclusive dates and duration of each experiment, the mean temperatures and the oxygen concentrations (means and ranges) recorded for the several test vessels in each experiment, the mean initial and final total lengths and wet and dry weights of the fish tested at each oxygen concentration, and the absolute and per cent increases in mean wet and dry weights of the fish. The mean initial lengths and weights shown are the mean lengths and weights of those fish that survived. In all experiments, there was a total loss of only seven fish; in no experiment did more than one fish die at any one oxygen concentration tested. In all cases but one, the initial weights of those fish that died could be reliably established and subtracted from the total initial weights of the fish in the test vessels in computing the mean initial weights of surviving fish. In experiment 2, the initial weight of a fish that died in vessel No. 5 could not be established, and it was assumed to have been equal to the mean initial weight of the 10 fish used in the test. The mean initial length of each group of surviving fish was computed in the same manner in which the mean initial weight was computed. The initial and final dates of each experiment shown in Table I are the date of the first feeding and the date on which the uneaten food from the last feeding was removed from the test vessels.

In Table II are given the total wet and dry weights of food consumed per surviving fish in each vessel during each test. In computing these values for a vessel in which a fish died, it was assumed that the fish that died consumed as much food per day up to the day of its death as did those fish that survived to the end of the test. The values computed on this basis may be slightly low, because the fish that were about to die probably did not feed as well as the surviving fish. However, the error cannot be large, since the mortality never exceeded 10% and the fish were initially nearly uniform in size.

Table II also includes, for each experiment, the average weight, in grams, of food consumed per day per gram of initial weight of the surviving fish in each vessel. Values based on both wet and dry weights of food and fish are given. Also included in Table II are the mean food conversion ratios, based on both wet and dry weights, for surviving fish in each vessel in which the fish gained weight. A food conversion ratio was computed by dividing the mean gain in weight of the fish in grams by the number of grams of food consumed per fish during the test, using either wet weights or dry weights.

GROWTH

The data in Table I indicate that when juvenile largemouth bass were held at temperatures near 26 C and fed unrestricted rations of small live earthworms, their growth was markedly influenced by the dissolved oxygen concentration. The per cent gain in dry weight for each group of fish tested at a constant oxygen concentration is plotted against the oxygen concentration in Fig. 2. A curve has been fitted by eye to the points representing the results of each separate experiment. Two different curves, designated A and B, have been fitted to the data from experiment 6, for reasons to be explained later. The per cent gains in dry weight, rather than gains in wet weight, were plotted in Fig. 2 because they are believed better to represent the relative amounts of consumed food substance that had been incorporated in the bodies of the fish.

TABLE I. Growth of surviving juvenile largemouth bass at various dissolved oxygen concentrations.

Expt. No. and test period (*1961*)	Test vessel No.	Mean temperature (*C*)	Dissolved oxygen (*mg/liter*)		Mean total length of fish (*mm*)		Mean initial weight of fish (*g*)		Gain in weight of fish (*g*)		Per cent gain in weight	
			Mean	Range[a]	Initial	Final	Wet	Dry[b]	Wet	Dry	Wet	Dry
Expt. 1	5	25.0	1.6	1.4–2.1	62.2	62.1	2.47	0.55	0.37	0.11	15	20
Apr. 10–25	2	25.1	2.3	1.9–3.8	61.8	68.4	2.58	0.58	1.54	0.37	60	64
(15 days)	4	25.2	3.0	2.7–3.8	61.7	72.4	2.65	0.59	2.50	0.64	94	108
	3	–	4.2	3.9–5.1	61.0	75.0	2.48	0.55	3.35	0.84	135	153
	1	25.1	5.8	5.5–6.4	63.1	76.1	2.71	0.60	3.75	0.94	138	157
	6	25.1	8.1	7.6–9.0	61.1	76.3	2.46	0.55	3.74	0.94	152	171
Expt. 2	5[c]	25.9	1.7	1.5–2.0	85.0	82.3	6.55	1.55	–0.49	–0.10	–7	–6
June 22–July 7	2	25.9	2.6	2.3–3.2	82.2	85.7	6.13	1.45	1.80	0.45	29	31
(15 days)	4	26.0	3.8	3.5–4.4	85.5	91.9	6.90	1.63	3.37	0.87	49	53
	3	–	5.4	4.9–5.9	85.7	96.4	6.90	1.63	4.57	1.18	66	72
	6	26.0	8.2	7.3–9.9	85.9	99.5	7.06	1.67	6.24	1.59	88	95
	1	26.0	1.8–3.7[d]	1.7–4.6	82.5	86.7	6.33	1.49	1.74	0.48	27	32
	7[c]	–	1.9–8.3[d]	1.6–10.5	85.4	92.3	6.85	1.62	3.02	0.80	44	49
Expt. 3	2	26.0	2.1	1.8–2.3	67.9	–	3.44	0.76	1.76	0.34	51	45
July 28–Aug 7	4[c]	26.0	3.4	3.2–3.5	67.5	–	3.50	0.77	2.89	0.64	83	83
(11 days)	3	–	5.9	5.6–7.0	65.3	–	3.13	0.69	3.92	0.86	125	125
	5[c]	26.0	10.0	9.6–10.9	65.7	–	3.21	0.70	3.48	0.85	108	120
	6	26.0	17.5	17.0–18.4	65.1	–	3.25	0.72	3.34	0.80	103	111
	1	26.0	2.1–5.9[d]	1.9–6.9	66.5	–	3.39	0.75	2.47	0.50	73	67
	7	–	2.1–17.4[d]	1.9–18.0	66.9	–	3.40	0.75	2.47	0.53	73	71

Expt. 4	6	26.1	2.1	1.9–3.4	67.7	76.2	3.36	0.76	1.83	0.43	54	57
Aug. 20–Sept. 4	3	–	3.4	3.0–3.8	68.4	82.8	3.42	0.77	3.59	0.81	105	105
(15.5 days)	4	26.1	5.9	5.6–6.9	69.3	85.4	3.52	0.80	4.29	1.02	122	128
	5	26.0	10.1	9.5–10.9	67.5	84.0	3.36	0.76	4.18	1.00	124	132
	2	26.0	17.2	8.8–18.4	68.0	83.9	3.36	0.76	4.00	0.96	119	126
	1	26.1	2.1–5.9[d]	1.8–6.6	67.5	79.7	3.32	0.75	2.72	0.63	82	84
	7	–	2.0–17.4[d]	1.8–18.4	68.7	80.8	3.48	0.79	2.46	0.57	71	72
Expt. 5	5[c]	25.7	1.9	1.8–2.8	73.8	80.0	4.16	0.93	2.10	0.56	50	60
Sept. 23–Oct. 8	2[c]	25.8	3.2	2.8–3.8	73.3	86.7	4.38	0.98	3.98	1.02	91	104
(15 days)	7	–	5.1	4.6–6.2	72.8	88.3	4.07	0.91	4.94	1.28	121	141
	1	25.8	8.0	6.8–8.9	73.4	92.7	4.29	0.96	6.50	1.67	152	174
	6[c]	25.8	24.1	23.3–27.0	73.2	87.0	4.11	0.92	4.18	1.11	102	121
	3	–	1.9–8.3[e]	1.8–10.7	72.2	82.4	4.11	0.92	2.96	0.75	72	82
	4	25.8	2.0–8.0[f]	1.8–9.9	73.7	86.4	4.42	0.99	3.78	0.98	86	99
Expt. 6	3	–	1.9	1.6– 2.8	64.5	71.5	3.39	0.84	1.26	0.30	37	36
Oct. 19–Nov. 3	4	25.6	3.8	3.5–4.6	64.0	76.4	3.25	0.80	2.88	0.68	89	85
(15 days)	6	25.6	8.1	7.4–9.2	64.1	77.9	3.19	0.79	2.79	0.65	87	82
	1	25.5	23.8	23.2–24.5	64.7	79.7	3.32	0.82	3.58	0.89	108	109
	7	–	3.8–24.1[d]	3.4–25.6	64.2	75.6	3.28	0.81	2.38	0.56	73	69
	2	25.5	1.8–8.4[e]	1.4–10.0	63.8	72.4	3.26	0.81	1.63	0.37	50	46
	5	25.6	1.8–8.1[f]	1.4–10.0	64.5	76.4	3.43	0.85	2.61	0.62	76	73

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[a] Range of observed values only.

[b] The mean initial dry weight was derived from the wet weight by using a factor (ratio of dry weight to wet weight) determined from a sample of 10 fish taken at the beginning of each experiment.

[c] One fish of the initial group of 10 fish died in the vessel during the experiment.

[d] The fish were subjected each day to oxygen concentrations near the lower and upper of the two values shown (mean low and mean high concentration extremes) for nearly equal periods.

[e] The fish were subjected each day to oxygen concentrations near the lower level shown (mean low concentration) about twice as long as they were subjected to concentrations near the upper level shown (mean high concentration).

[f] The fish were subjected each day to oxygen concentrations near the lower level shown (mean low concentration) about half as long as they were subjected to concentrations near the upper level shown (mean high concentration).

TABLE II. Food consumption and conversion by surviving largemouth bass at various dissolved oxygen concentrations.

Expt. No. and mean oxygen concentration (*mg/liter*)	Grams of food consumed per fish		Grams of food consumed per day per gram initial weight of fish[a]		Food conversion ratio[a,b]	
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight
Expt. 1						
1.6	4.77	0.81	0.129	0.098	0.078	0.136
2.3	9.46	1.61	0.244	0.185	0.163	0.230
3.0	14.04	2.39	0.353	0.270	0.178	0.268
4.2	17.42	2.96	0.468	0.359	0.192	0.284
5.8	19.54	3.32	0.481	0.369	0.192	0.283
8.1	19.02	3.23	0.515	0.392	0.197	0.291
Expt. 2						
1.7	7.54	1.28	0.077	0.055	–	–
2.6	16.03	2.73	0.174	0.126	0.112	0.165
3.8	23.09	3.93	0.223	0.161	0.146	0.221
5.4	29.52	5.02	0.285	0.205	0.155	0.235
8.2	34.93	5.94	0.330	0.237	0.179	0.268
1.8–3.7[c]	15.81	2.69	0.167	0.120	0.110	0.178
1.9–8.3[c]	20.84	3.54	0.203	0.146	0.145	0.226
Expt. 3						
2.1	8.47	1.44	0.224	0.172	0.208	0.236
3.4	12.07	2.05	0.314	0.242	0.239	0.312
5.9	16.27	2.77	0.473	0.365	0.241	0.310
10.0	15.55	2.64	0.440	0.338	0.224	0.322
17.5	15.35	2.61	0.429	0.330	0.218	0.307
2.1–5.9[c]	11.11	1.89	0.298	0.229	0.222	0.265
2.1–17.4[c]	10.97	1.86	0.293	0.225	0.225	0.285

Expt. 4						
2.1	10.90	1.85	0.209	0.157	0.168	0.232
3.4	17.87	3.04	0.337	0.255	0.201	0.266
5.9	20.76	3.53	0.380	0.285	0.207	0.289
10.1	21.08	3.58	0.405	0.304	0.198	0.279
17.2	19.83	3.37	0.381	0.286	0.202	0.285
2.1–5.9[c]	14.43	2.45	0.280	0.211	0.188	0.257
2.0–17.4[c]	13.98	2.38	0.259	0.194	0.176	0.239
Expt. 5						
1.9	15.26	2.59	0.245	0.186	0.138	0.216
3.2	25.88	4.40	0.394	0.299	0.154	0.232
5.1	28.73	4.88	0.471	0.358	0.172	0.262
8.0	36.65	6.23	0.570	0.433	0.177	0.268
24.1	25.33	4.31	0.411	0.312	0.165	0.258
1.9–8.3[d]	19.50	3.32	0.316	0.241	0.152	0.226
2.0–8.0[e]	24.12	4.10	0.364	0.276	0.157	0.239
Expt. 6						
1.9	10.23	1.74	0.201	0.138	0.123	0.172
3.8	16.97	2.88	0.348	0.240	0.170	0.236
8.1	18.24	3.10	0.381	0.262	0.153	0.210
23.8	20.93	3.56	0.420	0.289	0.171	0.250
3.8–24.1[c]	14.69	2.50	0.299	0.206	0.162	0.220
1.8–8.4[d]	11.03	1.88	0.226	0.155	0.148	0.197
1.8–8.1[e]	15.58	2.65	0.303	0.208	0.168	0.234

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[a] Values in the wet weight column and the dry weight column are based on wet weights of both fish and food and on dry weights of both fish and food respectively.

[b] Weight gain per fish in grams (see Table I) divided by the total weight of food consumed per fish in grams.

[c] See footnote *d* in Table I.

[d] See footnote *e* in Table I.

[e] See footnote *f* in Table I.

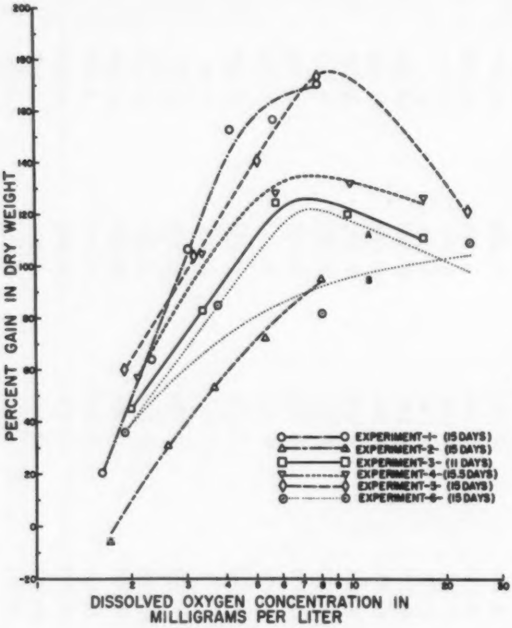

FIG. 2. Growth of largemouth bass in relation to dissolved oxygen concentration.

A difference in relative water content of the fish was observed. The fish held at low oxygen concentrations usually had a lower per cent dry weight than fish held at concentrations near the air-saturation level. This difference indicates a lower content of stored fats in the tissues of fish held at the lower oxygen concentrations, a phenomenon demonstrated in recent experiments with juvenile coho salmon, *Oncorhynchus kisutch*, by Fisher (MS, 1963).

Figure 2 shows that the per cent gain in dry weight of the fish generally increased with increase of oxygen concentration up to the air-saturation level, which is about 8.2 mg/liter at 26 C. The growth of fish held at oxygen concentrations well above the air-saturation level was generally depressed, as compared with growth at concentrations near the air-saturation level. The mean per cent weight gains of fish held for 15 days at nearly the same oxygen concentrations in different experiments varied greatly. For example, in experiment 1, fish held at mean oxygen concentrations of 1.6 and 8.1 mg/liter increased in dry weight by 20 and 173%, respectively, whereas in experiment 2, with much larger bass, the fish held at a mean oxygen concentration of 1.7 mg/liter decreased in dry weight by 6%, and fish held at a mean oxygen concentration of 8.2 mg/liter increased in dry weight by only 95%. It should be noted that the duration of experiment 3 (11 days) was much shorter than that of the other experiments. The weight gains of the fish at all the tested oxygen concentrations doubtless would have been much greater after 15 days than they were after 11 days.

The gain in weight of the fish held at a mean oxygen concentration of 8.1 mg/liter in experiment 6 was apparently much less than it should have been, since somewhat greater weight gains were observed at the next lower and higher concentrations tested. In all the other experiments, the growth at concentrations near 8 mg/liter was decidedly faster than growth at concentrations well below and far above this value. The cause of the relatively slow growth of the fish held at 8.1 mg/liter dissolved oxygen in experiment 6 is unknown. One of the two dotted curves in Fig. 2 that pertain to experiment 6, the one designated by the letter A, was drawn so that it would nearly parallel the curve based on the results of experiment 3. The latter curve seems to represent a typical relationship between dissolved oxygen concentration and the growth of juvenile largemouth bass, this curve being intermediate in shape between those fitted to the points representing the results of experiments 4 and 5. When a similar curve (A) was fitted to the data from experiment 6, the low observed per cent gain in weight of the fish held at a mean oxygen concentration of 8.1 mg/liter in this experiment was disregarded and it was assumed that the value obtained at 24 mg/liter dissolved oxygen was somewhat too high. The shape of this hypothetical curve may be inappropriate, since seasonal changes of the relationship between oxygen concentration and growth rate of the bass may occur; however, curve A probably represents better the true relationship than does the curve designated by the letter B, which has been fitted to all of the data from experiment 6 without reference to the results of the other experiments.

In Table III we have presented the per cent gain in dry weight of each group of fish that was held at diurnally fluctuating dissolved oxygen concentrations, the mean daily maximum and minimum oxygen concentrations for each of the tests, and the approximate arithmetic and geometric mean oxygen concentrations to which the fish were exposed throughout the tests. The latter values are arithmetic and geometric means of the mean upper and lower dissolved oxygen extremes, weighted or adjusted according to exposure time when the periods of exposure to low and high oxygen concentrations were not equal. Also given in Table III are the per cent gains in dry weight of bass that presumably would have occurred had the fish been held at constant levels of dissolved oxygen equal to the arithmetic and geometric mean oxygen concentrations computed for each test with fluctuating dissolved oxygen. These values were obtained by interpolation, using the appropriate curves in Fig. 2. For each listed set of experimental conditions tested in experiment 6, two such values are given in each of the last two columns of Table III, because two alternative curves have been fitted to the data from this experiment. The second or higher value of each pair was obtained from curve A (Fig. 2) and is believed to be more nearly correct than the preceding, lower value, which was taken from curve B and is almost surely less than the correct value.

TABLE III. Comparison of dry weight gains of bass subjected to fluctuating dissolved oxygen concentrations with the gains (estimated by interpolation) at constant oxygen concentrations corresponding to the means for the fluctuating dissolved oxygen tests.

Expt. No. and duration	Approximate (mean) limits of dissolved oxygen fluctuations (*mg/liter*)		Approximate mean dissolved oxygen concentration (*mg/liter*)[a]		Observed gain in dry weight[b] (%)	Estimated gain in dry weight (%)[c] at constant oxygen concentration equal to:	
	Mean low D.O.	Mean high D.O.	Arithmetic mean	Geometric mean		Arithmetic mean D.O.	Geometric mean D.O.
Expt. 2	1.8	3.7[d]	2.8	2.6	32	35	30
15 days	1.9	8.3[d]	5.1	4.0	49	74	59
Expt. 3	2.1	5.9[d]	4.0	3.5	67	96	86
11 days	2.1	17.4[d]	9.8	6.1	71	122	123
Expt. 4	2.1	5.9[d]	4.0	3.5	84	112	102
15.5 days	2.0	17.4[d]	9.8	6.0	72	133	132
Expt. 5	1.9	8.3[e]	4.0	3.1	82	122	101
15 days	2.0	8.0[f]	6.0	5.0	99	156	141
Expt. 6	3.8	24.1[d]	14.0	9.6	69	100–110	95–118
15 days	1.8	8.4[e]	4.1	3.0	46	74–91	61–68
	1.8	8.1[f]	6.0	4.9	73	86–115	80–103

[a] The approximate mean oxygen concentrations shown are weighted means (arithmetic and geometric means weighted according to exposure time) of the mean low and mean high concentrations given in preceding columns.

[b] Actual weight gain of the fish subjected to fluctuating dissolved oxygen.

[c] Weight gain estimates were derived from the appropriate curves in Fig. 2, including both curves A and B fitted to the data from experiment 6 (see text).

[d, e, f] See correspondingly designated footnotes in Table I.

The approximate arithmetic and geometric mean oxygen concentrations given in Table III for test vessels with fluctuating concentrations are believed to be slightly higher than the true mean concentrations. Arithmetic means based on eight series of dissolved oxygen determinations made every 2 hr in the course of 24-hr periods have been compared with approximate arithmetic means for the same 24-hr periods based only on the high and low concentrations that were regularly determined. The latter means were, on the average, greater by about 3% than the former (presumably true) means. The oxygen concentration in the test vessel usually approached the maximum or minimum level within about 4 to 5 hr after each change of the oxygen content of the influent water and remained more or less constant thereafter. The constant level apparently was approached somewhat less rapidly when the oxygen concentration was being increased than when it was being reduced. Because of inaccuracy of estimates of the concentration means, some of the estimates of dry weight gains that would have occurred at constant oxygen concentrations equal to the mean concentrations in vessels with fluctuating dissolved oxygen (Table III) may be somewhat high. These errors are believed to be negligible, however.

The data in Table III indicate that the growth of bass subjected alternately to low and higher dissolved oxygen concentrations was markedly impaired. It was almost always less than the growth that presumably would have occurred had the fish been held at a constant concentration equal to the mean concentration (either the arithmetic or the geometric mean) of the water with fluctuating oxygen content to which they had been exposed. The data from experiments 5 and 6 (Table III) further show that fish exposed for 8 hr of each day to relatively low oxygen concentrations and for 16 hr to higher concentrations grew more rapidly than did fish that were exposed for 16 hr of each day to the low concentrations and for 8 hr to the higher concentrations.

Food Consumption and Food Conversion

Figure 3 shows how the average food consumption rates of juvenile largemouth bass at various constant oxygen concentrations were related to the dissolved oxygen levels. A curve fitted by eye to the plotted points is shown for each experiment. In Fig. 3, the curve pertaining to experiment 6 was drawn to correspond in shape to the curve for experiment 3, and it is thus the counterpart of curve A in Fig. 2. No counterpart of curve B of Fig. 2 is included in Fig. 3. The effects of reduced and abnormally high oxygen concentrations on food consumption rates (Fig. 3) were similar to the effects on growth (Fig. 2). The observed differences in growth of the bass at different oxygen concentrations thus appear to be closely associated with differences in appetite.

The relation between oxygen concentrations and food conversion ratios, or gross conversion efficiencies, is shown in Fig. 4. The food conversion ratio for the fish held at a mean oxygen concentration of 1.7 mg/liter in experiment 2 was considered to be 0 in plotting the data, because these fish lost weight and a negative conversion ratio is not believed to be meaningful. In fitting a curve

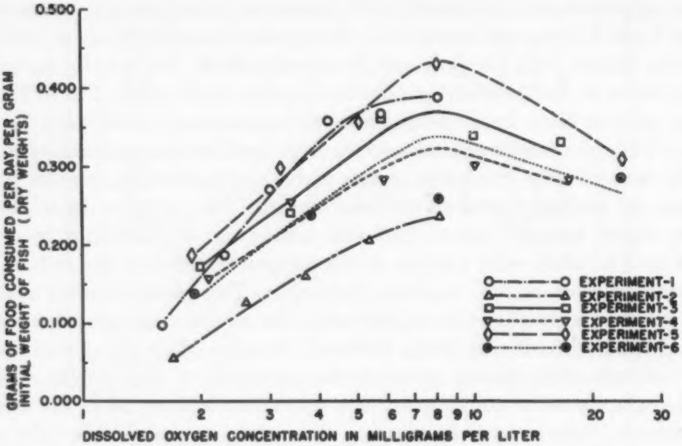

FIG 3. Food consumption rate in relation to dissolved oxygen concentration.

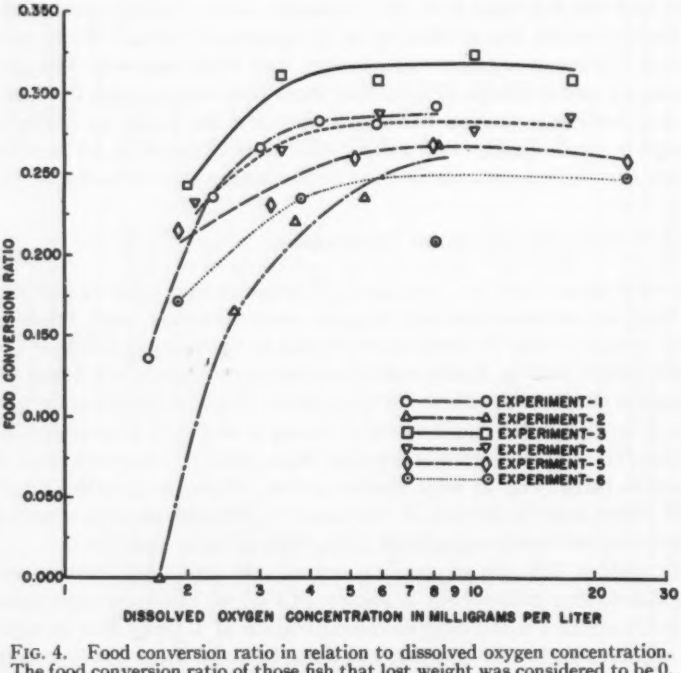

FIG. 4. Food conversion ratio in relation to dissolved oxygen concentration. The food conversion ratio of those fish that lost weight was considered to be 0, regardless of the weight loss.

to the data from experiment 6, we again disregarded the anomalous result obtained at the oxygen concentration of 8.1 mg/liter. The data plotted in Fig. 4 show that the gross food conversion efficiency was considerably reduced at oxygen concentrations well below 4 mg/liter. However, the efficiency does not increase very much with increase of oxygen concentration beyond 4 mg/liter. A slight reduction of food conversion efficiency may have occurred at oxygen concentrations far above the air-saturation level.

Only the shapes and not the relative positions of the individual curves shown in Fig. 3 and Fig. 4 can be meaningfully compared, because of the previously noted sources of error in the estimation of dry weights of food consumed. The curves in each figure probably would have been somewhat differently disposed in relation to each other had the ratio of dry weight to wet weight of the consumed food been determined accurately for each individual experiment. Undetermined and variable portions of the dry or wet weights of worms eaten are referable to the weights of residual contents of their alimentary tracts, of little or no nutritional value for fish. Nevertheless, the relationships between the food consumption or the conversion efficiency values obtained in any one experiment, with food of uniform quality, are certainly meaningful.

DISCUSSION

Largemouth bass have been shown by Moss and Scott (1961) to be quite tolerant of low oxygen concentrations. Yet, in our experiments, their appetite and growth were quite as markedly impaired by moderate reductions of dissolved oxygen from the air-saturation level, such as reductions to 50% of saturation, as were the appetite and growth of coho salmon in comparable tests (Herrmann et al., 1962; Fisher, MS, 1963; Doudoroff and Warren, 1965). The curves in Fig. 2 and 3 are sharply inflected only at oxygen concentrations near the air-saturation level, both higher and lower concentrations having had a depressing effect on food consumption and growth. The discrepant result of experiment 6 must be largely disregarded in arriving at these general conclusions.

The growth rates of the bass tested at nearly equal oxygen concentrations in different experiments varied widely, for reasons still undetermined. No consistent relation could be found between the growth rates and the initial weights of the fish, the initial length–weight relationships (condition indices), or the time of year when the experiments were performed. Groups of bass obtained from different sources may have differed genetically, but the fish used in experiments 3 and 4 (which were performed at about the same time of year) were obtained from the same source and their growth and food consumption rates still were markedly different. In experiment 3, the bass grew almost as much in 11 days as the bass from the same source used in experiment 4 did in 15.5 days (Fig. 2). Had experiment 3 been continued successfully for 15 days, the per cent gains in weight of the fish held at oxygen concentrations near the air-saturation level doubtless would have been much greater than those observed in experiment 4.

The reason for the reduced appetite and growth of bass held at oxygen concentrations well above the air-saturation level, as compared with appetite and growth at concentrations near the saturation level, is also uncertain. The toxicity of oxygen at high partial pressures to air-breathing warm-blooded animals is well known. West and Todd (1955) have pointed out that it may be due, at least in part, to impairment of transport by the blood and of elimination of carbon dioxide, resulting in an excess of carbon dioxide in extracellular and intracellular fluids of the body and in associated decrease of their *p*H. It may also be due to direct destruction or inhibition by oxygen of some enzyme systems (Stadie et al., 1944; Dickens, 1946). There was no evidence that the bass held in oxygen-treated water were afflicted with gas-bubble disease, which can occur when the sum of the tensions of dissolved atmospheric gases sufficiently exceeds the hydrostatic pressure (Doudoroff, 1957).

The food conversion ratios of largemouth bass were considerably reduced at oxygen concentration well below 4 mg/liter, whereas reduced concentrations near and above 4 mg/liter usually had very little effect on food conversion efficiency. Similar results have been obtained in experiments with coho salmon by Herrmann et al. (1962), who have discussed their significance. When the rate of food consumption is low, all or most of the consumed food may be needed for satisfying maintenance requirements, so that little or no conversion of the food to new tissue may be possible. On the other hand, digestive and assimilatory efficiencies may improve materially with reduction of the food consumption rate, even when this reduction is due to oxygen deficiency. The maintenance food requirement may or may not be increased when the oxygen concentration is reduced. An increase of the energetic cost of gill irrigation (due to increase in rate and amplitude of opercular movements) and any impairment of food digestion and of assimilatory or metabolic efficiency resulting from reduction of oxygen availability would tend to depress the efficiency of conversion of any constant food ration. However, their potential effect may be offset by that of reduction of the activity of the fish, since any muscular work can detract from food conversion efficiency. Complete understanding of the overall effect of a given reduction of dissolved oxygen on food conversion when rations are unrestricted can be achieved only through careful evaluation of all of the various possible efficiency-depressing and compensatory factors considered above.

The observed adverse effect of wide diurnal fluctuations of oxygen concentration on the growth of largemouth bass appears to be an important consideration in the definition of environmental conditions favorable for fish life. Regulatory agencies concerned with water pollution control often have prescribed only single minimal limits of dissolved oxygen concentration for the protection of desirable warmwater fish populations in waters receiving organic wastes. The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1955) has suggested, however, that dissolved oxygen concentrations in suitably protected warmwater fish habitats may be permitted to decline to relatively low nonlethal levels if much higher concentrations persist for the greater part of each 24-hr day. The value of this suggestion must now be questioned. The results of the present investigation indicate that even when oxygen concentrations are near or above saturation levels during a large portion of each day, the growth of fish may be much inhibited by very low concentrations occurring during the remainder of the day. Exceedingly high concentrations far above air-saturation levels occurring during daylight hours not only do not compensate for the occurrence of low concentrations at night and early in the morning, but also may do additional harm. Tarzwell and Gaufin (1953) have pointed out that average dissolved oxygen concentrations are by themselves of little value as indices of the suitability for fish of dissolved oxygen conditions in aquatic environments. The minimum and maximum levels evidently must be considered in evaluating fish habitats and the adverse effects of organic pollution not only because these extremes may be lethal. They can be more instructive than averages even when the ability of fish to survive is not in question.

Under natural conditions, the adverse effects of reduced oxygen concentrations on the growth of bass may be smaller or even greater than the effects observed under the experimental conditions. In the laboratory tests unlimited food was available and could be ingested with little effort. In nature, the availability of food may often be the principal factor limiting the rates of food consumption and growth even when oxygen concentrations are moderately low. A limited amount of food consumed may be digested and assimilated as efficiently at the reduced oxygen concentrations as at high concentrations. However, if the activity of the fish at the low oxygen concentrations is restricted so much that less food is found and captured, reduction of the growth rate is likely to occur. The per cent reductions of food intake and growth rates that will result from reduced feeding activity at given low oxygen concentrations in nature, where food availability is limited, are as yet unpredictable. They may be greater or less than the reductions due entirely or chiefly to impaired appetite that occur at the same oxygen concentrations in laboratory aquaria with abundant food. This matter has been more fully discussed by Doudoroff and Warren (1965).

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REFERENCES

AQUATIC LIFE ADVISORY COMMITTEE OF THE OHIO RIVER VALLEY WATER SANITATION COMMISSION. 1955. Aquatic life water quality criteria — First progress report. Sewage Ind. Wastes, **27**: 321–331.

BOUCK, G. R., AND R. C. BALL. 1965. Influence of a diurnal oxygen pulse on fish serum proteins. Trans. Am. Fish. Soc., **94**: 363–370.

DICKENS, F. 1946. The toxic effects of oxygen on brain metabolism and on tissue enzymes. 2. Tissue enzymes. Biochem. J., **40**: 171–187.

DOUDOROFF, P. 1957. Water quality requirements of fishes and effects of toxic substances. *In* M. E. Brown [ed.] Physiology of fishes. Vol. II. Academic Press, Inc., New York. p. 403-430.

1960. How should we determine dissolved oxygen criteria for fresh water fishes? *In* Biological problems in water pollution: Transactions of the 1959 seminar. Tech. Rept. W60–3. Robert A. Taft Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, Ohio. p. 248–250.

DOUDOROFF, P., AND C. E. WARREN. 1965. Dissolved oxygen requirements of fishes. *In* Biological problems in water pollution: Transactions of the 1962 seminar. Public Health Serv. Publ. 999–WP–25. Ibid. p. 145–155.

FISHER, R. J. MS, 1963. Influence of oxygen concentration and of its diurnal fluctuations on the growth of juvenile coho salmon. M.Sc. Thesis. Oregon State University.

HAEMPEL, O. 1928. Über die Wirkung höherer Sauerstoffkonzentration auf Fische nebst Untersuchungen über die Ausnützung des künstlich ins Wasser eingeleiteten O_2. Z. Vergleich. Physiol., **7**: 533–570.

HERRMANN, R. B., C. E. WARREN, AND P. DOUDOROFF. 1962. Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Am. Fish. Soc., **99**: 155–167.

MOSS, D. D., AND D. C. SCOTT. 1961. Dissolved oxygen requirements of three species of fish. Ibid., **90**: 377–393.

STADIE, W. C., B. C. RIGGS, AND N. HAUGAARD. 1944. Oxygen poisoning. Am. J. Med. Sci., **207**: 84–114.

TARZWELL, C. M., AND A. R. GAUFIN. 1953. Some important biological effects of pollution often disregarded in stream surveys. *In* Proceedings of the eighth industrial waste conference. Purdue University Eng. Bull. Ser. No. 83. p. 295–316.

WEST, E. S., AND W. R. TODD. 1955. Textbook of biochemistry. 2nd ed. Macmillan, New York. 1356 p.

WIEBE, A. H. 1931. Diurnal variations in the amount of dissolved oxygen, alkalinity, and free ammonia in certain fish ponds at Fairport, Iowa. Ohio J. Sci., **31**: 120–126.

1933. The effect of high concentrations of dissolved oxygen on several species of pond fishes. Ibid., **33**: 110–126.

WIEBE, A. H., AND A. M. MCGAVOCK. 1932. The ability of several species of fish to survive on prolonged exposure to abnormally high concentrations of dissolved oxygen. Trans. Am. Fish. Soc., **62**: 267–274.

WOODBURY, L. A. 1941. A sudden mortality of fishes accompanying a supersaturation of oxygen in Lake Waubesa, Wisconsin. Ibid., **71**: 112–117.

Reproductive Isolation Between the Sunfish *Lepomis gibbosus* and *L. macrochirus*[1,2]

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ABSTRACT

Reproductive isolation appeared to be complete between pumpkinseeds (*Lepomis gibbosus*) and bluegills (*L. macrochirus*) when equal numbers (1:1 sex ratio) were stocked in each of two similar ponds near London, Ontario, and observed during the breeding seasons of 1964 and 1965. Ethological isolation, through visual recognition of potential conspecific mates, was probably the major barrier to hybridization. Interspecific courtship and attempted spawning were not seen; intraspecific courtship and spawning were frequently observed. No clearly identifiable hybrids were found among large numbers of yearling and 2-year-old offspring collected in both ponds in 1965 and 1966.

Pumpkinseeds began nesting about 3 weeks before bluegills, but spawning by the two species broadly overlapped in time both years. Individual pumpkinseeds occupied nests for an average of 18.5 days; bluegills for 8.7 days. Duration of pumpkinseed nest occupancy appeared to vary directly with surface water temperature, whether eggs or fry were in the nests or not, whereas bluegills tended to remain on nests containing eggs or fry, regardless of temperature fluctuations. Bluegills nested in deeper water and further from shore than pumpkinseeds, and bluegills tended to nest in colonies whereas pumpkinseeds did not. Interspecific differences were not found in nest site selection with respect to substrate or vegetation.

INTRODUCTION

THIS STUDY concerns reproductive isolation between two sunfish species (Family Centrarchidae), the pumpkinseed (*Lepomis gibbosus* (L.)) and the bluegill (*L. macrochirus Rafinesque*). The ranges of these species broadly overlap in eastern North America (Trautman, 1957) and hybrids between them are frequently found (Bailey and Lagler, 1938; Ball and Tanner, 1951). They have also been successfully interbred in the laboratory (Hubbs and Hubbs, 1932). Centrarchid hybridization in nature has been associated with: (*a*) competition for suitable spawning areas; (*b*) dissimilar numbers of two species; or (*c*) introduction of one species into an area containing another (Hubbs, 1955). This paper describes an experiment in which pumpkinseeds and bluegills were stocked in two ponds under conditions which eliminated the above three factors. It

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[2] This paper is based on a thesis submitted by F. W. Clark to The University of Western Ontario in partial fulfillment of the requirements for the degree of M.Sc.

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was then possible to compare the relative effectiveness of seasonal, habitat, and ethological isolating mechanisms (Mayr, 1963) between the two species.

Bailey (1938) suggested that: "In general the time of breeding and the choice of nesting sites among closely related species of centrarchids are subject to such broad overlapping that these factors are not barriers to hybridization." He implied that behavioral incompatibilities are the most important barriers. Our experiment was designed to test these statements and to gather evidence on the means by which behavioral barriers to interspecific breeding operate.

MATERIALS AND METHODS

The two study ponds, named Blackburn and White, are situated on gravel soil on glacial outwash terraces, above the present floodplain of the Thames River, Middlesex County, Ontario. The surrounding district is open farmland, with scattered deciduous and coniferous woodlots, and extensive gravel quarries. Both ponds have constant-flow springwater inlets and vertical standpipe outlets which stabilize water levels throughout the year. Plane-table maps were made of each pond, and depth contours were plotted by grid survey with a transit and other surveying equipment. During the study predominant vegetation was stonewort, *Chara* sp., which covered most of the bottom of both ponds except for a narrow strip around the edge. Other aquatic vegetation included: cat-tails, *Typha* sp.; arrowhead, *Sagittaria* sp.; white waterlily, *Nymphaea* sp.; pondweed, *Potamogeton* sp.; duckweed, *Lemna* sp.; and unidentified filamentous algae. The bottom was gravel overlain by silt varying in depth from none at the shoreline to nearly a meter in deeper areas.

Blackburn Pond is located in unused pasture land, with several small willow trees, *Salix* sp., and woody shrubs near its border. The surface area is 0.11 ha and maximum depth is 1.5 m. The bottom drops sharply away from the shoreline in some areas, gradually in others. It contained no fish prior to the experiment.

White Pond is surrounded by well-kept lawn, with weeping willow trees, shrubs, and ornamental plants close to its border. The surface area is 0.25 ha and maximum depth is 1.4 m. A few speckled trout, *Salvelinus fontinalis* (Mitchill), were in the pond during the experiment.

Pumpkinseeds were captured with a beach seine in October 1963, in Pinehurst Lake, near Galt, Ontario, and planted the same day. Bluegills were obtained from a commercial trap net set under the ice at Mitchell's Bay, Lake St. Clair, Ontario, January 28, 1964, and planted 2 days later. They were probably *L.m. macrochirus* (Trautman, 1957). More fish were stocked in White than in Blackburn Pond because the former is larger and had more potential spawning area. The numbers and mean lengths and weights of fish stocked in the two ponds were as follow.

Pond	Length (*cm*)	Weight (*g*)	Number measured	Number stocked
		Pumpkinseeds		
White	11.4 ± 1.17[a]	28.0 ± 8.69	52	150
Blackburn	11.2 ± 1.06	21.9 ± 7.73	27	100
		Bluegills		
White	16.4 ± 1.88	96.7 ± 37.64	50	150
Blackburn	16.3 ± 2.23	96.5 ± 50.46	50	98

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[a] Standard deviation.

A separate sample of each species was preserved for aging and sexing. Numbers of fish in various age-classes in these samples were as follow.

Age	3+	4+	5+	6+	
Pumpkinseeds	11	10	3	1	Total 25
Bluegills	10	7	3	0	Total 20

Ages were determined by scale examination, a method validated for pumpkinseeds by Creaser (1926) and for bluegills by Regier (1962).

The sex of each fish was estimated by examination of shape and color of the body, fins, and opercular flaps (Trautman, 1957). Sex was then determined by macroscopic or microscopic study of the gonads.

The ratios (♂ : ♀) were as follow.

	Ratio		% sexed correctly	Number examined
	Estimated	Actual		
Pumpkinseeds	54:54	61:47	84.2	108
Bluegills	14:39	29:24	56.6	53

Chi-square analysis (Snedecor, 1956) revealed a difference between estimated and actual sex ratios of bluegills ($P < .005$) but not of pumpkinseeds ($P > .10$). We concluded that in fall and winter we could sex pumpkinseeds, but not bluegills, fairly accurately by external features. Chi-square analysis showed no difference ($P > .10$) between the actual sex ratio of either species and a 1:1 ratio, and therefore a 1:1 sex ratio in the fish stocked was assumed.

On several occasions in 1964 samples of both species were captured in Blackburn Pond by seining or angling, marked for later identification, and returned to the pond. The basic marker was a yellow rectangular plastic tag with rounded corners. It was 14 mm long and 8.5 mm wide and was attached by a double strand of stainless steel wire (0.30 mm diam) just anterior to the first dorsal spines. The tags were either cut into different shapes or painted. On calm days, using binoculars with polaroid filters, individual fish could be recognized to a maximum distance of about 8 m. Nesting activity of tagged fish in Blackburn Pond was studied in detail during 1965.

Twenty-one bluegills and 18 pumpkinseeds were found dead in Blackburn Pond between April 3 and May 10, 1965. Of these, 15 bluegills and 13 pumpkinseeds had been tagged. Death apparently resulted from a fungus disease (*Saprolegnia* sp.). All dead fish and several living ones had white patches of fungus on the head, caudal peduncle, fins, and unhealed tag wounds. No fungus growth was seen on fish in White Pond. On May 6, 1965, 27 bluegills and 30 pumpkinseeds were transferred from White to Blackburn Pond to raise the latter's population level. No dead fish were found in Blackburn Pond after May 10, and fish observed later appeared to be free of fungus. No other known mortalities occurred in either pond during the study.

Pond Observations

From the time of ice break-up in the spring of 1964 until sunfish nesting had ceased in mid-August, both ponds were visited almost every day. Surface, inlet, and outlet water temperatures were taken, and general weather conditions were recorded at each visit. A thermograph was installed on May 16 at Blackburn Pond and another on June 22 at White Pond. On every visit two or three circuits of each pond were made (10–30 min total time) and the following activities of sunfish were recorded: basking (staying virtually motionless just beneath the water surface), nest-building, territorial behavior, and spawning. When first seen, each nest was assigned a number and its location was plotted on a sketch map of the pond. Nests were classified as occupied, if a male fish was present on the nest, or vacant. The presence of eggs or fry in a nest was also recorded.

During the spring and summer of 1965, the same observations were made again 1964, but at several times of day according to a random sampling schedule. Dates and times of observation were selected from an 84-day observation period, April 18 to July 10. This period was divided into four blocks of 21 days each, and summer daylight hours (arbitrarily designated as 3 AM to 10 PM EST) were divided into four equal time periods. Observations were made near the middle of a period, i.e. at 5 AM, 10 AM, 3 PM, or 8 PM. In each block of 21 days, 28 observation periods were selected randomly, with the restriction that equal numbers of observations be made in each of the four time periods. In 1965 each sunfish nest in Blackburn Pond was identified by an anchored 4-cm ^{2} styrofoam marker, labelled with India ink.

The following measurements were made at all nest sites in both years: depth of water over nest centre, shortest distance from nest centre to shore, distance from nest centre to centre of nearest conspecific nest, and distance from nest centre to nearest vegetation. Each nest was also classified according to type of substrate in its centre. In 1964 these measurements were made on several occasions between June 4 and October 7 using a meter stick and steel tape measure. Some nests were not measured because they were obscured by silt stirred up while wading. In 1965 all measurements were made on July 12 and 13 with the same equipment but while swimming with skin-diving gear.

Thermograph records of surface water temperatures at the two ponds in 1964 were compared. Data from 53 days when both instruments were functioning (inadequate weatherproofing caused the White Pond thermograph to stop occasionally) were subjected to a *t*-test analysis (Snedecor, 1956). The sample mean difference between ponds was 0.52 C, standard error ±0.10 C. Although the *t*-test indicated a significant difference ($P < .001$), this is probably within the limits of accuracy obtainable when setting and reading the thermographs (Dickson Minicorder Type 2A at White; Taylor Type L at Blackburn) and hence should be disregarded. Furthermore, analysis of variance on the same data did not show a significant difference ($P > .05$). On these grounds the difference in water temperature between ponds was not considered meaningful and records from the Blackburn Pond thermograph were used for both ponds in both years. Temperatures at the surface and at 50-cm and 100-cm depths in Blackburn Pond were so similar throughout the summer of 1965 that only the surface temperatures were used for analysis.

When the experiment ended in July 1965, both ponds were seined thoroughly and several hundred yearling sunfish retained for identification. Yearling and 2-year-old fish were also collected in both ponds in June and September 1966. These fish were aged by scale examination. Differences in shape and color of body and fins, color and flexibility of opercular flaps, and shape of gill rakers and pharyngeal teeth are commonly used to distinguish pumpkinseeds from bluegills (Trautman, 1957). All of these characteristics were examined in samples of these juvenile sunfish from both ponds. Underyearlings, when captured, were considered too small for accurate identification.

RESULTS

In both years of this study sunfish were seen in the ponds as soon as ice-cover melted in the spring. They stayed in deep water in schools apparently containing both species and sexes, although identification from the shoreline was difficult. As water temperatures rose, male pumpkinseeds were the first to leave the schools and move singly into shallow water near the pond edges. They often stayed near shore for several days before beginning nest-building; bluegill males remained in deep water until the day they began nesting.

Pumpkinseeds began nesting in both ponds in early May in 1964 (Fig. 1, 2). The number of occupied nests increased quickly, remained at a high level until mid-June, then rapidly declined. Nest occupancy by bluegills began about 3 weeks later in both ponds and remained relatively constant until nesting ceased in late July.

The same interspecific differences in nesting activity were observed in 1965 (Fig. 3 and 4). Pumpkinseeds began nesting 2 or 4 weeks earlier than bluegills

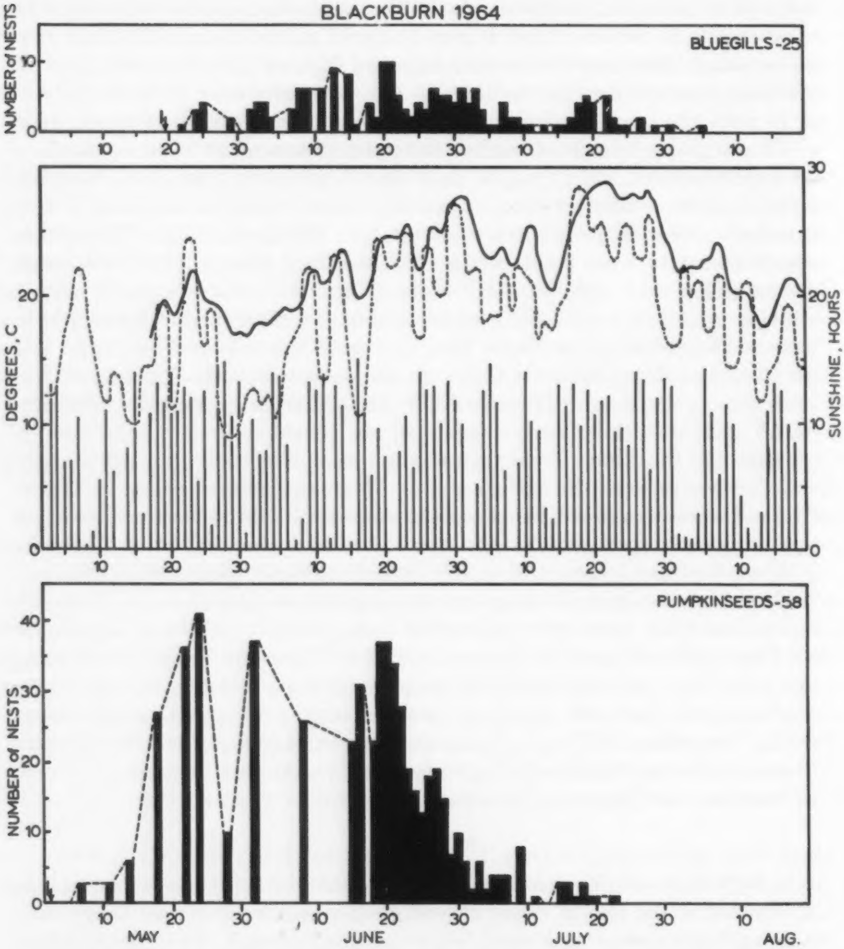

Fig. 1. Numbers of nests occupied, mean air and water temperatures, and hours of sunshine, daily, Blackburn Pond, 1964. Areas under dotted lines in histograms, no observations made. Solid curve, surface water temperature; broken curve, air temperature; vertical lines, hours of bright sunshine. Small arrow, date when nests first observed if prior to date of first nest seen occupied. Number with species name, total nests observed occupied. Air temperature and sunshine date from Weather Bureau, London, Ontario.

and the nest occupancy graphs for pumpkinseeds show a more pronounced peak than do those for bluegills. Although equal numbers of the two species were stocked in each pond, many more pumpkinseeds than bluegills built nests in both years.

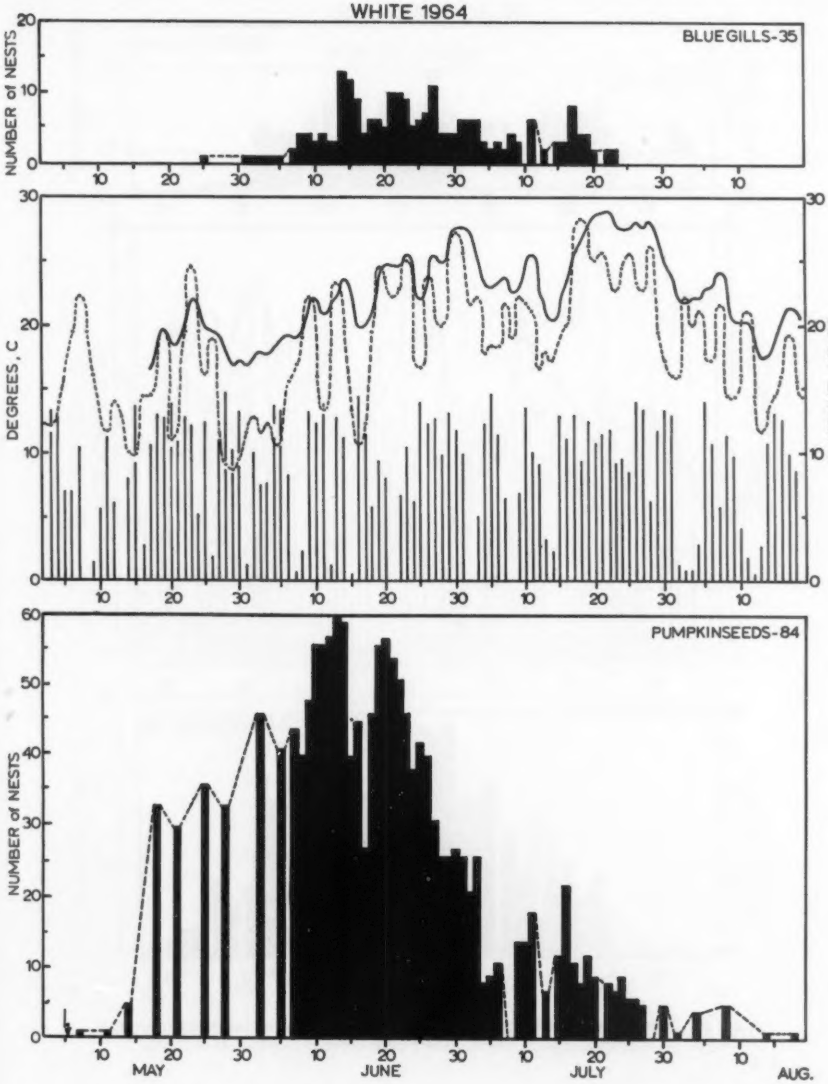

FIG. 2. Numbers of nests occupied, mean air and water temperatures, and hours of sunshine, daily, White Pond, 1964. Legend as for Fig. 1.

The relation between number of occupied nests and surface water temperature appears to be different in the two species. In 1965 pumpkinseed nests were first seen in White Pond when the mean daily surface water temperature was 13 C and in Blackburn Pond when it was 17 C (Fig. 3 and 4). Comparable

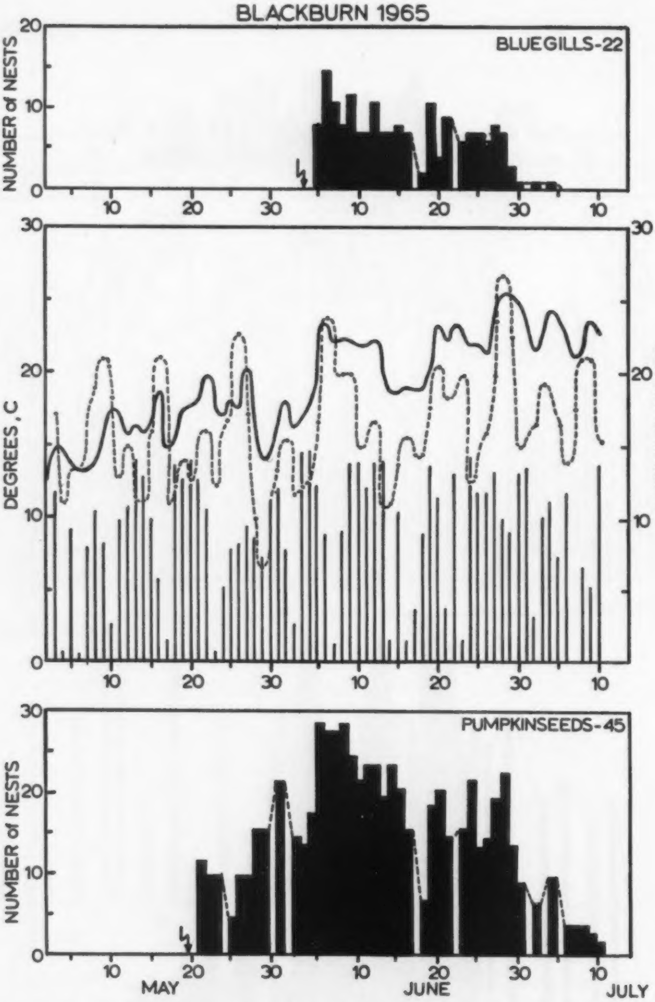

FIG. 3. Numbers of nests occupied, mean air and water temperatures, and hours of sunshine, daily, Blackburn Pond, 1965. Legend as for Fig. 1.

figures for bluegills are 23 and 17 C. Similar data are unavailable for this period in 1964 because thermographs were not then in operation. Visual inspection of the graphs suggests that once nests were established, numbers of occupied pumpkinseed nests varied directly with mean daily surface water temperature (Fig. 1–4). Numbers of bluegill nests occupied did not demonstrate this relationship as clearly. Increases in numbers of occupied bluegill nests appeared

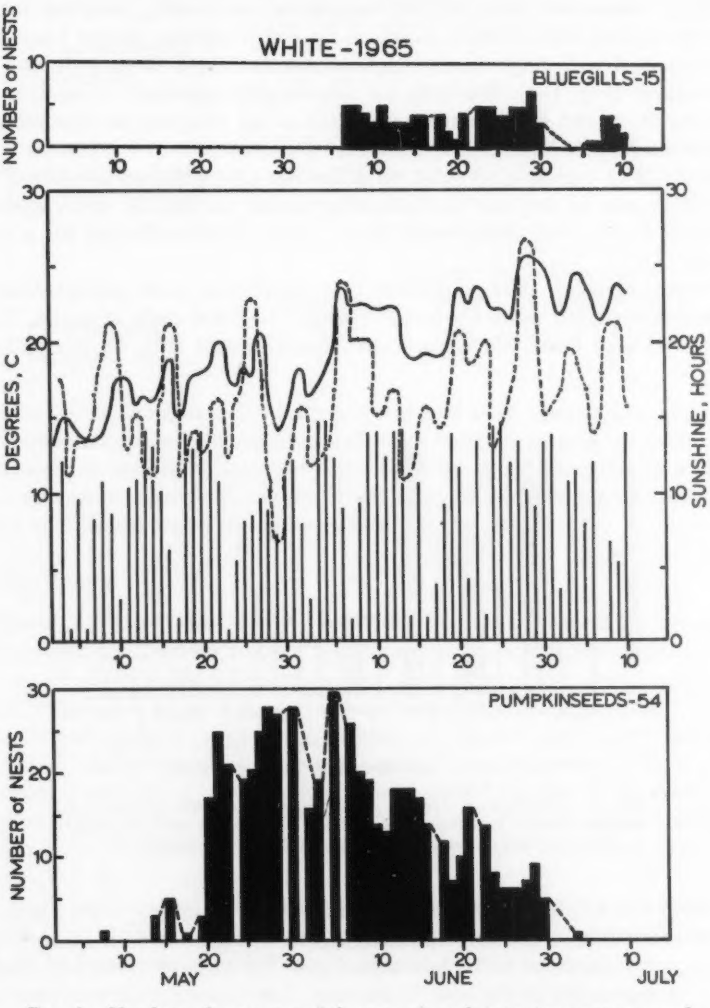

FIG. 4. Numbers of nests occupied, mean air and water temperatures, and hours of sunshine, daily, White Pond, 1965. Legend as for Fig. 1.

to correspond with rising surface water temperatures, but a relationship between falling water temperature and nest occupancy was not always evident. Correlation analysis of these data was considered unsuitable because the relation between nesting activity and water temperature changed during the breeding season for both species.

Visual inspection also did not suggest a relationship between numbers of occupied nests and hours of sunshine for either species, except when bright days coincided with high water temperatures and dark days with low water temperatures (Fig. 1–4). Similarly no relationship appeared to exist between air temperature and nest occupancy, except as air temperature was related to water temperature.

Analysis of variance revealed no difference ($P > .05$) in number of nests occupied in any of the four daytime observation periods by either species in Blackburn Pond, 1965. Data from White Pond were insufficient for a similar analysis.

Observations in 1964 suggested that individual male pumpkinseeds in both ponds occupied nests for longer periods than did male bluegills. Tagged fish in Blackburn Pond, 1965, confirmed this difference (Fig. 5). For pumpkinseeds the mean number of consecutive days spent guarding a nest was 18.5 (SD 12.8); for bluegills the corresponding figure was 8.7 (SD 5.0). Pumpkinseed nest occupancy appeared to be determined more by water temperature than by presence of eggs or fry in the nest. Swimming observations confirmed that some pumpkinseeds deserted nests containing eggs when water temperature fell suddenly; others stayed on nests without eggs for over 3 weeks with no sudden temperature drop. Bluegill nest occupancy was determined primarily by presence of eggs or fry in the nest. Some bluegills stayed on nests containing eggs through periods of fluctuating water temperature; those without eggs usually deserted their nests within 4 days of initial occupancy.

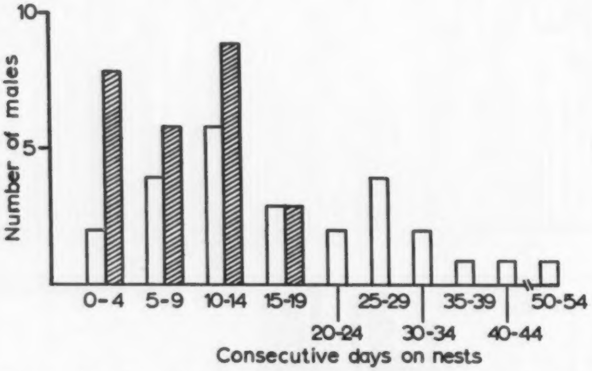

FIG. 5. Numbers of tagged male sunfish observed nesting for various periods, Blackburn Pond, 1965. Open bars, pumpkinseeds; hatched bars, bluegills. Total number for each species, 26.

Tagged males in Blackburn Pond also provided information on fidelity of individual fish to nests. The numbers of tagged fish observed occupying one, two, or three different nests in 1965 were as follow.

	Number of different nests occupied		
	1	2	3
Pumpkinseeds	12	5	1
Bluegills	14	5	1

The numbers of nests occupied by tagged males relative to the builders of those nests were as follow.

	Nest-builder		
	Occupant	Conspecific male	Heterospecific male
Pumpkinseeds	6	6	0
Bluegills	14	5	2

These two tables show that some tagged males of both species occupied more than one nest, that some males occupied nests built by conspecific males, and that bluegills occasionally occupied nests built by pumpkinseeds. In addition to these data from tagged fish, several nests, especially in Blackburn Pond, were occupied at different times by males of both species (Fig. 6–8).

NEST LOCATIONS

Figures 6–9 show locations of nests built by pumpkinseeds and bluegills, including nests occupied by males (tagged and untagged) of both species. These maps suggest interspecific differences in nest sites with respect to depth of water, distance from shore, grouping, and overhanging shoreline vegetation.

Depth and distance from shore of nests of the two species are compared in Fig. 10. Bluegills nested in deeper water than pumpkinseeds in both ponds, both years (*t*-test, $P < .01$). Bluegills also nested further from shore than pumpkinseeds ($P < .01$), except in White Pond, 1965. This exception was caused by high variance introduced by four pumpkinseed nests built about 10 m offshore (see Fig. 9).

Bluegill nests tended to be grouped, whereas pumpkinseed nests were more scattered (Fig. 6–9). The mean distances to the nearest conspecific nesting neighbour in 1964 were as follow.

	White Pond			*Blackburn Pond*		
	Distance (*cm*)	Number of nests	Number measured	Distance (*cm*)	Number of nests	Number measured
Pumpkinseeds	60 ± 32[a]	84	60	54 ± 17	58	35
Bluegills	50 ± 19	35	9	32 ± 12	25	14

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[a] Standard deviation.

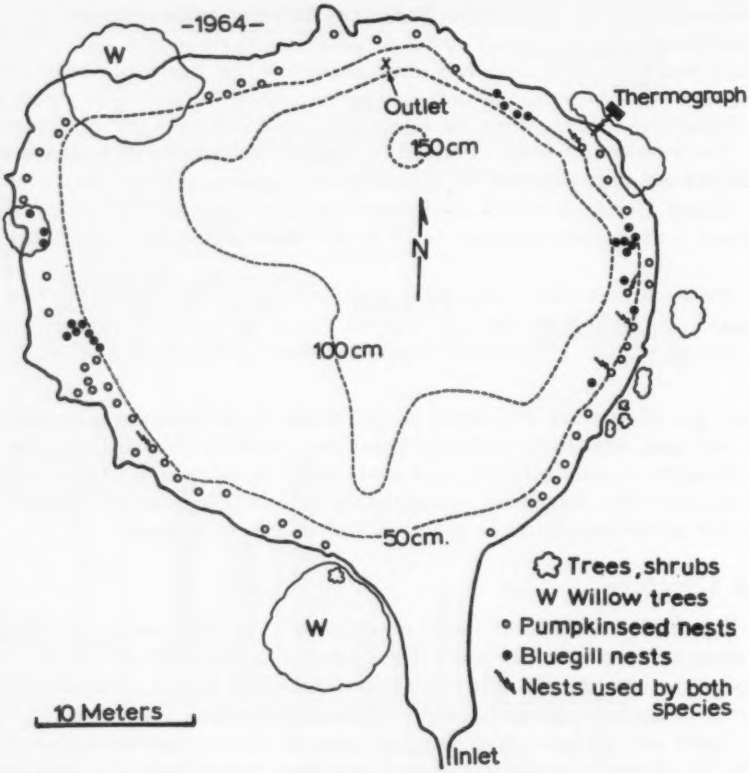
-1964-
W
Outlet
150cm
N
100cm
50cm.
Thermograph
W
10 Meters
Inlet
Trees, shrubs
W Willow trees
Pumpkinseed nests
Bluegill nests
Nests used by both species

FIG. 6. Blackburn Pond showing depth contours, locations of sunfish nests, 1964, and other salient features.

Corresponding data for 1965 were as follow.

	White Pond			Blackburn Pond		
	Distance (*cm*)	Number of nests	Number measured	Distance (*cm*)	Number of nests	Number measured
Pumpkinseeds	210±97	54	41	143±78	45	45
Bluegills	99±30	15	15	69±17	31	31

t-Test analyses of the above data revealed a significant difference ($P < .001$) between species in all cases except White Pond, 1964 ($.20 < P < .40$).

Shade and cover from trees overhanging the water did not appear to influence nest-site selection by pumpkinseeds in either pond. In White Pond most bluegill nests were under or close to shade from large willows (Fig. 7 and 9).

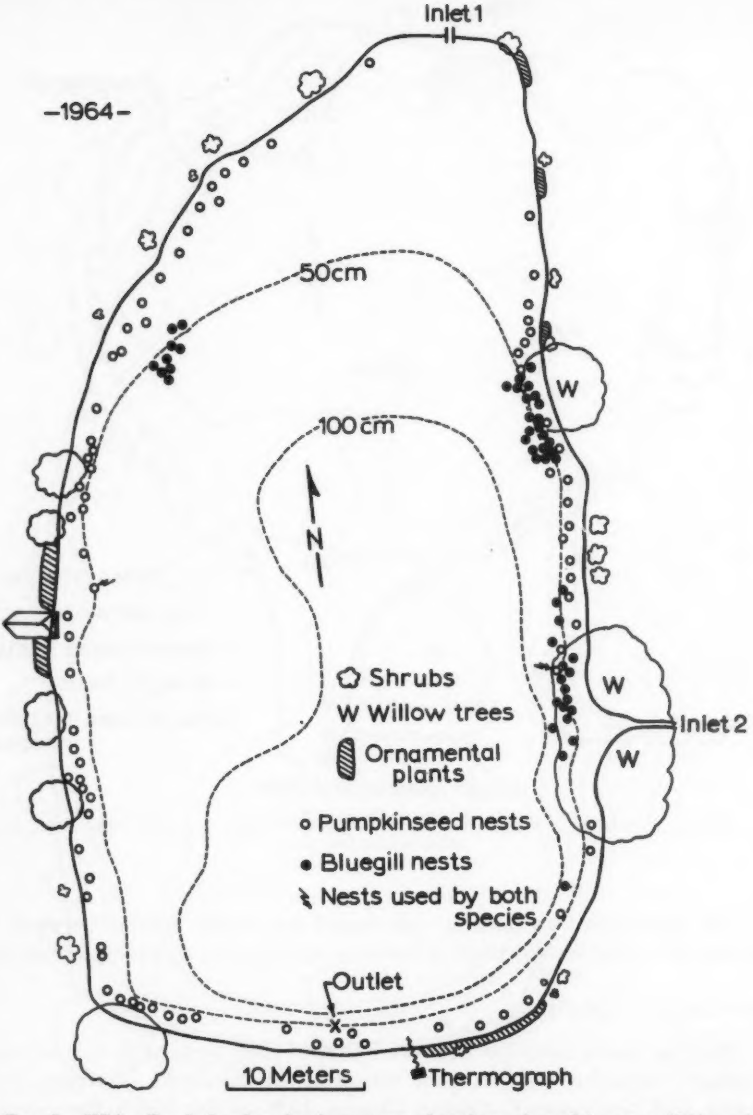
-1964-
Inlet 1
50cm
100cm
N
W
W
W
Inlet 2
🟢 Shrubs
W Willow trees
▨ Ornamental plants
○ Pumpkinseed nests
● Bluegill nests
Nests used by both species
Outlet
10 Meters
Thermograph

FIG. 7. White Pond showing depth contours, locations of sunfish nests, 1964, and other salient features.

Similar shade areas were not present in Blackburn Pond, where branches of one willow leaned into the water but left no space for nesting underneath. Few bluegill nests in Blackburn Pond were built near trees or shrubs (Fig. 6 and 8).

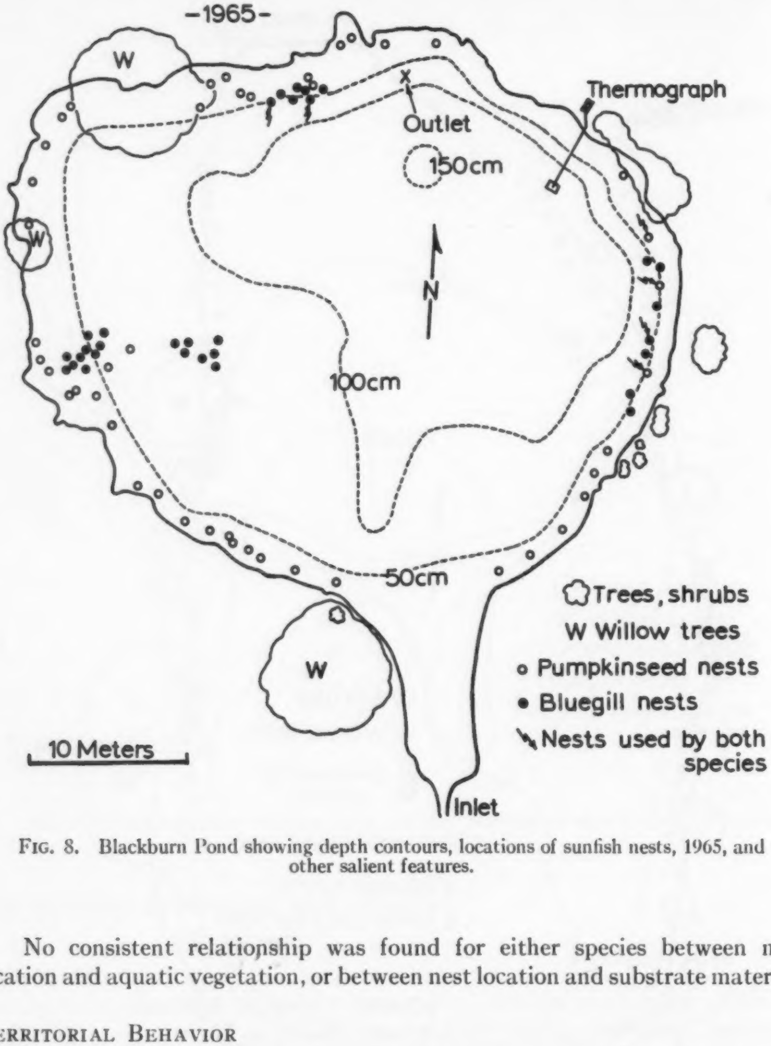
-1965-
W
W
W
Outlet
Thermograph
150cm
N
100cm
50cm
Trees, shrubs
W Willow trees
○ Pumpkinseed nests
● Bluegill nests
Nests used by both species
10 Meters
Inlet

FIG. 8. Blackburn Pond showing depth contours, locations of sunfish nests, 1965, and other salient features.

No consistent relationship was found for either species between nest location and aquatic vegetation, or between nest location and substrate material.

TERRITORIAL BEHAVIOR

Nesting males defended territories around their nests with typical sunfish aggressive behavior, consisting of spreading the opercula, charging, biting, chasing, and, rarely, mouth-fighting (Breder, 1936; Miller, 1963). Pumpkinseeds maintained larger territories than bluegills. Approaching fish of either species were often attacked by nesting pumpkinseeds when they were about 1 m from the nest. Nesting bluegills seemed to defend only the nest itself, seldom attacking an intruding fish until it was within a few centimeters of the nest. Males of both species showed aggression initially towards any approaching female.

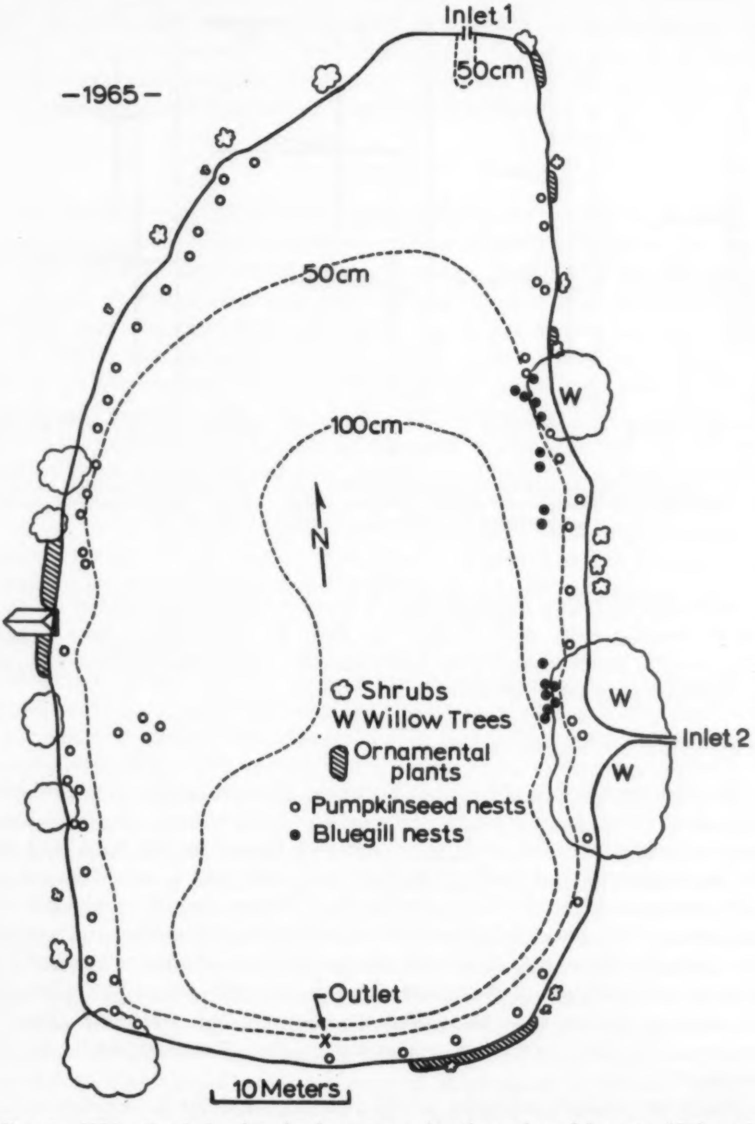
Inlet 1
50cm
-1965-
50cm
100cm
N
W
W
W
Inlet 2
Shrubs
W Willow Trees
Ornamental plants
Pumpkinseed nests
Bluegill nests
Outlet
10 Meters

FIG. 9. White Pond showing depth contours, locations of sunfish nests, 1965, and other salient features.

Subsequent behavior of the female appeared to determine the outcome of these encounters. A mature female threatened by a nesting conspecific male either fled or settled closer to the bottom and withstood his attack; in the latter case,

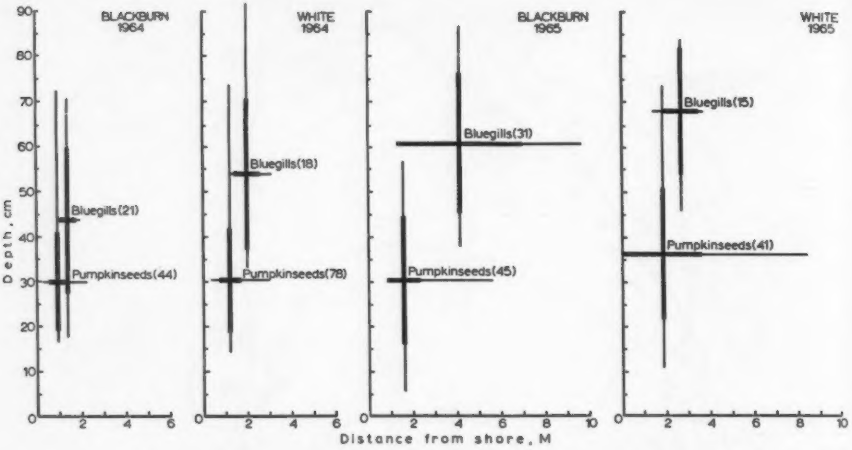

FIG. 10. Depths and distances from shore of sunfish nests. Numbers in brackets, numbers of nests measured; thin vertical and horizontal lines, ranges; thicker lines, standard deviations; intersections of vertical and horizontal lines, means.

the male usually began courting her. A mature female approached by an aggressive heterospecific male invariably fled. Females were never observed trying to enter nests occupied by heterospecific males, and interspecific courtship and spawning were not seen.

EXAMINATION OF YOUNG SUNFISH

Yearling and 2-year-old sunfish collected in both ponds were tentatively identified as bluegills or pumpkinseeds on the basis of body shape and color. Those classed as pumpkinseeds were yellowish-brown on the back and sides, with prominent vertical barring on the sides, and had a small orange spot on the posterior edge of each opercular flap. Those classed as bluegills were greyish-blue on the back and sides, with indistinct lateral barring and with solid black opercular flaps. Relatively greater lateral compression of the body and a more sharply pointed snout characterized the bluegill group. The color differences between groups were most distinct when the fish were still alive, and assortment into the two species groups was confirmed independently by three observers.

Wherever possible, samples of 50 yearlings and 50 2-year-olds of each tentatively identified group, collected in each pond in 1966, were studied in more detail. Fewer than 50 fish were available in the yearling bluegill group from White Pond (11) and in the 2-year-old bluegill group from Blackburn (7). Internal examination supported the tentative identifications. The bluegills had relatively long, thin gill rakers; long, narrow pharyngeal bones; and thin, sharp pharyngeal teeth. The pumpkinseed group had shorter, thicker gill rakers; broader and heavier pharyngeal bones; and their pharyngeal teeth were blunter and more molar-like. These are all features used to distinguish bluegills from pumpkinseeds (Trautman, 1957) and on this basis none of the juvenile fish examined from either pond was considered to be a hybrid.

DISCUSSION

The absence of clearly identifiable pumpkinseed–bluegill hybrids in the two ponds is strong evidence that reproductive isolation between the species was complete. Identification of the mechanism(s) responsible for this isolation was a major objective of this study. Reproductive isolating mechanisms have generally been classified into two major types: pre-mating and post-mating (Mayr, 1963). The latter involves gamete or zygote mortality, hybrid inviability or hybrid sterility. None of these appears to be important in bluegill–pumpkinseed crosses (Luce, 1937; Lagler and Steinmetz, 1957), although they were not investigated in the present study. Pre-mating isolation may be maintained by three means: (*a*) potential mates do not meet (i.e. seasonal or habitat isolation); (*b*) potential mates meet but do not mate (ethological isolation); and (*c*) copulation is attempted but no transfer of sperm takes place (mechanical isolation) (Mayr, 1963). Since fertilization is external in the Centrarchidae, mechanical isolation between these species does not exist.

SEASONAL ISOLATION

Although many authors suggest the breeding seasons of bluegills and pumpkinseeds are almost identical, our results show that pumpkinseeds began nesting about 3 weeks before bluegills (Fig. 1–4). The start of nest-building was associated with rising surface water temperatures in the spring, supporting Breder's (1936) hypothesis that water temperature controls initiation of sunfish reproduction. In our study a higher absolute temperature or a faster rise in temperature, or both, appeared to be necessary to start bluegill nesting than pumpkinseed nesting. Pumpkinseeds began nesting at temperatures from 13 to 17 C, and bluegills at temperatures from 17 to 23 C. These are similar to temperatures at which the two species have been reported to begin spawning elsewhere (Breder, 1936; Mraz and Cooper, 1957; Snow et al., 1960; Miller, 1963). Water temperatures in the two ponds rose more gradually in the spring of 1965 than 1964 and more pumpkinseeds occupied nests early in the season, before any bluegill nesting occurred, than in 1964. This interspecific seasonal difference in initiation of nesting was most pronounced in White Pond, 1965 (Fig. 4). In spite of such differences, much spawning by both species occurred during the same period in each pond, both years. Seasonal isolation between them was therefore considered unimportant.

HABITAT ISOLATION

The interspecific differences in nesting locations in the two ponds do not suggest that habitat isolation played a major role in preventing hybridization.

Nest sites of both species did not appear to be influenced by proximity of aquatic vegetation or by type of substrate. Bluegills and pumpkinseeds often occur together in ponds and lakes, where they commonly nest on gravel or sand bottoms (e.g. Ball and Tanner, 1951) or on whatever substrate type is most available (Carbine, 1940). Our observations support Carbine's conclusion. Bluegills generally nested in deeper water and further from shore than pumpkinseeds (Fig. 10); the latter, with few exceptions, nested close to shore even when equally shallow areas existed well offshore, as in the north end of White Pond (Fig. 7 and 9). Also, most bluegill nests in White Pond were concentrated near overhanging willow trees (Fig. 7 and 9). However, the distances between nests of the two species were not great in either pond and, in addition, several nests were successively occupied by males of both species.

Thus, observed habitat isolation seems inadequate to explain the apparent effectiveness of reproductive isolation. Females of both species spent most of their time in the deeper central water of each pond. Sexually mature females were seen cruising through shallow waters where they must have encountered males of both species. These interactions could only lead to hybridization if ethological isolating barriers were inadequate.

ETHOLOGICAL ISOLATION

If incompatible reproductive behavior patterns prevented interspecific breeding in the two ponds, at least two separate mechanisms may have been involved: nesting males may have courted conspecific females but rejected or ignored heterospecific females, and gravid females may have distinguished between the two types of males. Some observational data are available on both mechanisms. Bluegill males frequently began nest-rim circling (Miller, 1963) on the approach of bluegill females, but ignored or attacked pumpkinseed females. Nesting male pumpkinseeds attacked all approaching females but chased conspecifics most actively. On the few occasions that female bluegills were seen approaching nesting male pumpkinseeds they quickly retreated when attacked, but they stayed near nesting male bluegills, especially when several were nest-rim circling together. Female pumpkinseeds were not seen near bluegill nests but persistently tried to approach pumpkinseed nests.

We have no data on the species recognition cues involved in these differential responses. Noble (1934) suggested male pumpkinseeds recognize females by their behavior in the nest. He elicited typical spawning behavior in a nesting male by manipulating models (some only slightly resembling a female pumpkinseed) in the nest to mimic a spawning female. Our observations show that male pumpkinseeds and bluegills responded differently to approaching conspecific and heterospecific females, and thus they were able to discriminate between them even before they entered a nest. Unpublished data (Miles H. A. Keenleyside) show that in aquaria mature male pumpkinseeds and bluegills presented with the two types of females predominantly court conspecific females. The recognition cues for this response, however, are not established.

Recognition of conspecific males by females could also ensure reproductive isolation. Male bluegills are easily distinguished from male pumpkinseeds by the human observer (see e.g. Harlan and Speaker, 1956) and possibly also by the female fish.

The frequency with which centrarchid hybridization is associated with extreme crowding of spawning areas (Bailey and Lagler, 1938; Hubbs, 1955) suggests that under severe competition for nesting sites, some males are unable to establish nests, and a large number of females will crowd into the nesting area and compete for potential conspecific mates. The species "recognition" that seems to have occurred under the conditions of our experiment might then break down. Nesting males would be unable to prevent some heterospecific females from entering their nests, and hybridization could then follow.

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REFERENCES

BAILEY, R. M. 1938. A systematic revision of the centrarchid fishes, with a discussion of their distribution, variations and probable interrelationships. Ph.D. Thesis. Univ. Michigan. 256 p.

BAILEY, R. M., AND K. F. LAGLER. 1938. An analysis of hybridization in a population of stunted sunfishes in New York. Papers Mich. Acad. Sci., **23**: 577–604.

BALL, R. C., AND H. A. TANNER. 1951. The biological effects of fertilizer on a warm-water lake. Tech. Bull. Mich. State Coll. Agr. Exptl. Sta., **223**: 32 p.

BREDER, C. M., JR. 1936. The reproductive habits of the North American sunfishes (Family Centrarchidae). Zoologica, **21**: 1–48.

CARBINE, W. F. 1940. Observations on the spawning habits of centrarchid fishes in Deep Lake, Oakland County, Michigan. Trans. 4th N. Am. Wildlife Conf. p. 275–287.

CREASER, C. W. 1926. The structure and growth of the scales of fishes in relation to the interpretation of their life-history, with special reference to the sunfish *Eupomotis gibbosus*. Misc. Publ. Museum Zool. Univ. Mich., No. 17. 82 p.

HARLAN, J. R., AND E. B. SPEAKER. 1956. Iowa fish and fishing. Iowa State Conserv. Comm., Des Moines, Iowa. 377 p.

HUBBS, C. L. 1955. Hybridization between fish species in nature. Syst. Zool., **4**: 1–20.

HUBBS, C. L., AND L. C. HUBBS. 1932. Experimental verification of natural hybridization between distinct genera of sunfishes. Papers Mich. Acad. Sci., **15**: 427–437.

LAGLER, K. F., AND C. STEINMETZ, JR. 1957. Characteristics and fertility of experimentally produced sunfish hybrids, *Lepomis gibbosus* and *L. macrochirus*. Copeia, 1957(4): 290–292.

LUCE, W. M. 1937. Hybrid crosses in sunfishes. Trans. Illinois State Acad. Sci., **30**: 309–310.

MAYR, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, Mass. 797 p.

MILLER, H. C. 1963. The behavior of the pumpkinseed sunfish *Lepomis gibbosus* (Linnaeus), with notes on the behavior of other species of *Lepomis* and the pigmy sunfish, *Elassoma evergladei*. Behaviour, **22**: 88–151.

MRAZ, D., AND E. L. COOPER. 1957. Reproduction of carp, largemouth bass, bluegills, and black crappies in small rearing ponds. J. Wildlife Management, **21**: 127–133.

NOBLE, G. K. 1934. Sex recognition in the sunfish, *Eupomotis gibbosus* (Linné). Copeia, 1934(4): 151–155.

REGIER, H. A. 1962. Validation of the scale method for estimating age and growth of bluegills. Trans. Am. Fish. Soc., **91**: 362–374.

SNEDECOR, G. W. 1956. Statistical methods. 5th ed. Iowa State Univ. Press, Ames, Iowa. 534 p.

SNOW, H., A. ENSIGN, AND J. KLINGBIEL. 1960. The bluegill, its life history, ecology and management. Publ. Wisconsin Conserv. Dept. No. 230. 16 p.

TRAUTMAN, M. B. 1957. The fishes of Ohio. Ohio State Univ. Press, Columbus, Ohio. 683 p.

Morphology of *Phrixocephalus cincinnatus* Wilson, 1908 (Copepoda: Lernaeoceridae)[1,2]

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ABSTRACT

The morphology of *Phrixocephalus cincinnatus* Wilson, 1908 (Copepoda: Lernaeoceridae) is described and discussed, with special reference to the structure of the antennae, hitherto almost unknown. Mention is also made of the mode of attachment and of its possible effect on the host.

INTRODUCTION

Phrixocephalus cincinnatus Wilson, 1908, is a lernaeocerid parasite, well known in the waters of British Columbia. Commonly found embedded in the eyes of its equally common flatfish host, *Atheresthes stomias* (Jordan and Gilbert, 1880), it has been frequently observed and collected by the members of the staff of the Biological Station in Nanaimo. Occurring also in the species of the flatfish genus *Citharichthys* Bleeker, 1862, it is known to extend along the coast of North America from British Columbia to California.

Phrixocephalus cincinnatus is the type species of the genus, which consists at present of 12 species. Eight of them were described by Shiino (1956) from Japanese waters. Yet another species, *P. cepolae* Yamaguti, 1939, has been recently transferred by its discoverer (Yamaguti, 1963) to a new genus, *Phrixocephaloides*. The parasites of the genus *Phrixocephalus* are all attached to their hosts by means of profusely branching antlers, which form an anchoring holdfast of great complexity. The definitive shape of the holdfast clearly depends on the conditions at the individual sites of attachment, but it has been used (Shiino, 1956) as one of the specific discriminants.

Most of the species of *Phrixocephalus* are known from few specimens only. Even the most common of them, *P. cincinnatus*, has never been described with sufficient accuracy. In particular, the appendages of this parasite are known only slightly. This is especially true of the antennae.

It is the purpose of this paper to describe the morphology of *P. cincinnatus*, its appendages in particular. They were studied from formalin-preserved specimens. The appendages were dissected out, cleared, and mounted in polyvinyl lactophenol. No stains were employed. Magnifications up to ×450 and phase contrast illumination were used throughout. All drawings were made with the aid of the eyepiece graticule.

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[2] This work was done at the Fisheries Research Board of Canada's Biological Station, Nanaimo, B.C., during the author's visit in the summer of 1966 while on leave from the Marine Laboratory, Aberdeen, Scotland; the author is now employed at the Nanaimo Station.

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ATTACHMENT OF THE PARASITE AND ITS EFFECTS ON THE HOST

When observed on its host, *P. cincinnatus* appears as a large, subcylindrical body, connected anteriorly to the eye of the host by a narrow, neck-like peduncle. Near the point of entry of the peduncle, the corneal surface is sometimes broken by a short, branched, heavily cuticularised outgrowth. The outgrowth is part of a subsidiary attachment organ, developed as lateral branches of the neck, halfway between the terminal holdfast and the base of the neck (Fig. 1, 9, and 10). The terminal part of the neck enters the eye of the host and traverses its entire width, the holdfast spreading usually in the chorioid layer of the fundus of the eye. In his description of the attachment of *Phrixocephalus*, Shiino (1956) drew attention to the considerable degree of involvement of the retina and doubted if the affected eye could be used effectively as an organ of vision. He found, however, that the presence of the parasite had no effect on the lens of the eye. The present author, in the material he examined, found that the lens of the affected eye was invariably surrounded by a thick capsule of opaque fibrous tissue. It appears, therefore, that the infestation of the eye with *P. cincinnatus* produces total blindness. Not infrequently, *A. stomias* is found with multiple infestations of both eyes. As many as five parasites have been seen by the author in one eye.

It is difficult to assess the effect of blindness on the host fish without resorting to experiment. *Atheresthes stomias* feeds on herring and shrimp (Clemens and Wilby, 1961), the capture of which appears difficult without the full use of sight. Most of the infested fish, collected on occasion by the scientists at Nanaimo, are about 10 inches long or less. The fish is capable, however, of reaching a length of 2 ft 9 inches (Clemens and Wilby, 1961). The author has been informed by Dr S. Wilkes (personal communication) that in the catches of *A. stomias* in Oregon, large fish are rarely found infested with *P. cincinnatus*. One might infer, therefore, that growth of the infested fish is impeded due to their inability to feed as effectively as the uninfested fish. It is, indeed, possible that in some instances at least the infestation has fatal consequences.

DESCRIPTION OF *P. CINCINNATUS*

The externally protruding part of the adult female has the appearance of a cylindrical trunk, somewhat wider posteriorly (Fig. 2). In the centre of the posterior margin there is the abdominal tubercle, lateral to which are the openings of the oviducts. The egg sacs, emerging from these orifices, are coiled in a spring-like fashion and filled with single rows of flat eggs. Anteriorly, the trunk narrows abruptly to form the peduncular neck (Fig. 1), which is round in cross section and gradually enlarges towards the point where it gives off two branches. These branches divide distally and expand into subsidiary anchoring processes. At the same level, on the ventral aspect of the neck are found two short outgrowths (Fig. 1, 9, and 10), which occur as a typical feature in all the species of the genus so far described. It is at this level also, that the fourth pair of thoracopods is situated in some species of *Phrixocephalus*. Directly proximal to the terminal holdfast, the neck of *P. cincinnatus* carries yet another and more slender pair of subsidiary anchoring processes. The presence of these processes provides the distinguishing feature of *P. cincinnatus*, which appears to be the only species of the genus equipped with this additional pair.

The differentiation of the body of the female into the trunk and neck parts is apparent only in the completely developed specimens. The already modified, but not yet ovigerous, female is shown in Fig. 10. A similar stage has been shown by Wilson (1922) for *P. diversus*. It can be seen that the part of the trunk concerned with reproduction develops later than that concerned with the more immediate needs of nutrition and attachment ('diphasic growth': Kabata, 1959 [1960]).

The trunk of the adult female often exhibits some degree of torsion, due to which the lateral aspect of the trunk is shown in Fig. 1 together with the dorsal aspect of the neck. Shiino (1956) described this phenomenon in more detail.

Just anterior to the smaller pair of the subsidiary anchoring processes the neck produces the main holdfast, which expands laterally around its circumference. Since the shape of the holdfast is conditioned by its association with the wall of the eyeball, it can be described as an umbrella, spreading at right angles to the axis of the neck. The holdfast (Fig. 3) consists of several main trunks, arising on both lateral aspects of the neck. There are usually four or five on each side. The antlers may divide very near the base or may grow out, giving off side shoots at intervals. The division is dichotomous and produces a maze of interlacing branches. The dorsalmost antlers, which are often more slender than the remaining ones, move towards each other and often cross over the dorsum of the neck.

In single infestations the holdfast might develop into a luxuriant canopy. When more than one parasite is present in the same eye, however, the overcrowding restricts the development of the holdfast. It seems feasible to suppose that under such conditions, the over exploitation of the eye has deleterious effects also on the nutrition of the parasite.

Anterior to the holdfast the neck comes to a rounded end. It is surmounted terminally by a group of papilliform processes, between and around which the antennae and the mouth parts are situated. In the present species these processes, or lobes, are fairly simple in comparison with such species as *P. umbellatus*, *P. curvatus*, *P. longicollum*, or *P. crassum* (cf. Shiino, 1956). It is not surprising, therefore, that the study of these species did not contribute much to the knowledge of the appendages.

The lobes (Fig. 4–6) comprise two pairs and one single lobe. The first of the two pairs are the anterior lobes (*al*, Fig. 4–8). These lobes are hollow structures, arising from fairly narrow peduncles and expanding in the dorso-ventral plane. They are bifid, with a third, smaller outgrowth on the ventral aspect close to the base (Fig. 8). The bases of the posterior lobes (*pl*, Fig. 4–7) are ventro-lateral to those of the anterior lobes. The posterior lobes differ from the anterior pair in being more deeply bifid and devoid of the small ventral outgrowth. They are farther apart from each other than the anterior lobes and enclose between them the single medial lobe (*ml*, Fig. 4–7). The medial lobe projects ventrally, is not subdivided, and bears on its anterior aspect the mouth-cone and the adjacent mouth parts (Fig. 7). In some specimens, but not all, particularly in those at more mature stages of development, the dorsal aspect of the cephalothoracic area, directly behind the bases of the anterior lobes, is occupied by a pair of small swellings (*ds*, Fig. 4).

THE APPENDAGES

The first antenna is very small and situated on both sides of the dorsal swellings (*ant′*, Fig. 6 and 7). It is cylindrical, indistinctly segmented, with only the terminal segment separated by a constriction from the remainder of the limb. As in other Lernaeoceridae, the first antenna of *P. cincinnatus* has along its anterior margin a number (about 15 in the specimens examined by the author) of naked, fairly robust, and slightly curved setae of varying length (Fig. 12). These setae are difficult to study, as they are very easily damaged by handling and cannot be examined in detail without dissecting the first antenna. In addition, slightly farther away from the anterior margin of the limb and near one-third of its length from the base, there are two more setae, immediately recognisable by the absence of the sabre-like curve common in the remaining setae. Closer examination shows that these two straight setae are provided with two rows of very short and widely spaced hairs. In this they resemble the corresponding structures of *Lernaeolophus* (Kabata, 1967a) and *Haemobaphes* (Kabata, 1967b). There are no setae on the anterior margin of the terminal segment. The arrangement and the composition of the apical armature of this segment are similar to those of the above-named genera. The most obvious structure (perhaps homologous with the aesthete of Gooding and Humes (1963)) is the apical, robust, and long seta with a finger-like tip (1, Fig. 11). Close to it is seta 2, slightly shorter and more slender. The characteristic seta 3 divides into two at about half its length and forms branches of unequal length. This is also a feature characteristic of the lernaeocerid first antenna in general. The further elements of the apical armature are three pairs of slender, shorter setae (pairs 4, 5, and 6) around the perimeter of the apex. Finally, there are also three single and much smaller setae, 7, 8, and 9, arising near the bases of the large setae, 2, 1, and 3 respectively.

FIG. 1–6: *Phrixocephalus cincinnatus*, female.

FIG. 1. General view of the trunk, peduncular neck in dorsal view and the genital segment in lateral view.

FIG. 2. Posterior part of the trunk, dorsal.

FIG. 3. Anterior extremity and holdfast, anterior.

FIG. 4. Frontal lobes, anterior.

FIG. 5. Frontal lobes, ventral.

FIG. 6. Frontal lobes, lateral; *al*, anterior lobe; *ant′*, first antenna; *ds*, dorsal swelling; *ml*, medial lobe; *pl*, posterior lobe.

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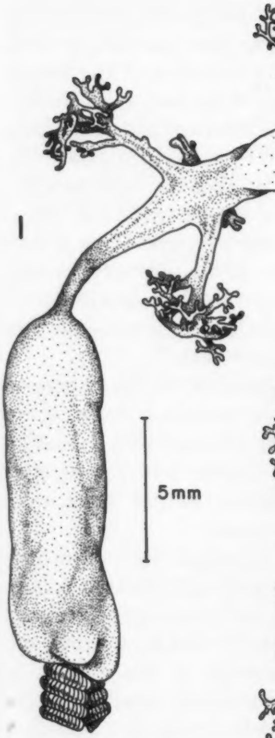

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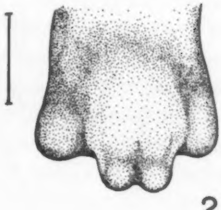

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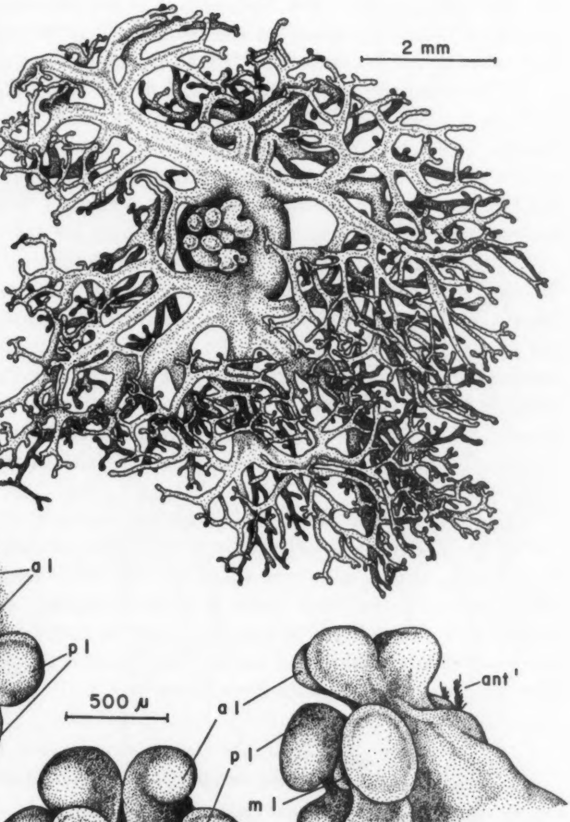

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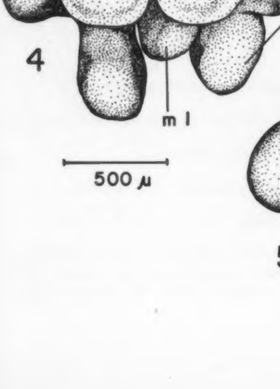
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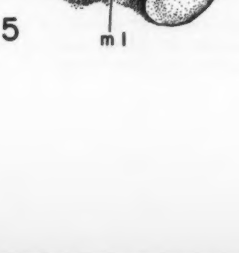
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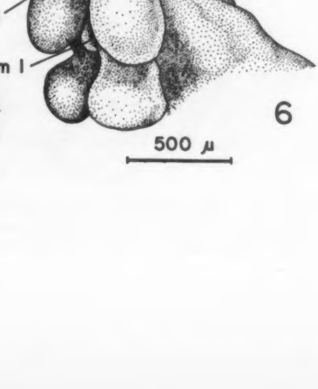


The second antenna (Fig. 8 and 13) arises from the inner aspect of the peduncle of the anterior lobe (*ant''*, Fig. 7 and 8). The lobe expands so as to cover the limb and hide it from view completely. The structure of the second antenna (Fig. 13) resembles that of other lernaeocerid genera. The limb is three-segmented. The basal segment is strongly cuticularised and articulates with the cephalothorax through a very mobile joint. It has no armanent. The middle segment articulates distally with the claw, formed by the terminal segment. When closed, the curving point of the claw enters the small pit at the base of a tooth-like outgrowth on the middle segment, providing a lock capable of efficient prehension. The author was unable to discover any secondary armanent on the claw, although it is not uncommon for Lernaeoceridae to possess a secondary spine on the proximal half of the claw. It is possible that such a spine will be eventually found in less mature specimens.

The structure of the buccal tube of *Phrixocephalus*, first shown in general outlines by Shiino (1956), conforms in the main to the general pattern in the family. The intrabuccal stylets (Fig. 14), shown in the drawing with a portion of the roof of the buccal cavity, are fairly long and slender and tipped with flexible setae. The author could not ascertain whether second setae were present at their tips, as in *Haemobaphes* and *Lernaeolophus*.

The mandible (Fig. 15) is attached on the lateral aspect of the mouth tube (*mt*, Fig. 7). It is a blade-like structure, apparently consisting of two parts. The distal part tapers to a narrow tip, rounded in outline and seemingly devoid of the usual dentiferous margin. The author was unable to decide whether the absence of the mandibular teeth is a normal phenomenon in this species, or whether it was the result of damage, either in vivo or through handling of the appendage, which is difficult to find and dissect. It is worth noting that a similar, toothless mandible has been described by the author for *Lernaeolophus* (cf. Kabata, 1967a).

The first maxilla (Fig. 16) is attached ventrally to the base of the mandible (*mx'*, Fig. 7). The main body of the first maxilla consists of a laterally compressed, short blade, which is surmounted by two long, finely tapering setae. On the outer aspect of the limb there is a small papilla, bearing a short and more slender seta. Both the first maxillae and the mandibles are wedged in between the rounded lateral slopes of the medial lobe and the walls of the posterior lobes. They are effectively hidden from observation.

FIG. 7–12. *Phrixocephalus cincinnatus*, female.

FIG. 7. Diagram showing distribution of the appendages on the anterior end.

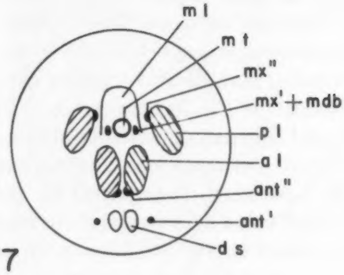
FIG. 8. Diagram showing lateral view of anterior lobe, with position of the second antenna.

FIG. 9. Larger auxiliary anchoring processes, ventro-lateral.

FIG. 10. Young, modified female; anterior part ventral, posterior part lateral.

FIG. 11. First antenna, tip, posterior view.

FIG. 12. First antenna, entire (apical armament not shown in detail), anterior; *al*, anterior lobe; *ant'*, first antenna; *ant''*, second antenna; *ds*, dorsal swelling; *mdb*, mandible; *ml*, medial lobe; *mx'*, first maxilla; *mx''*, second maxilla; *mt*, mouth-tube; *pl*, posterior lobe; *th*, thoracic legs.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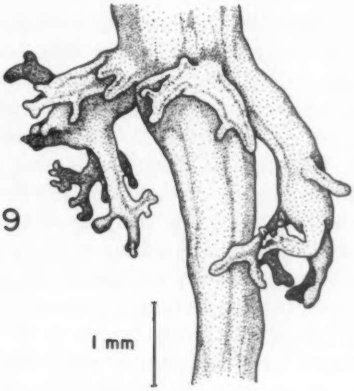
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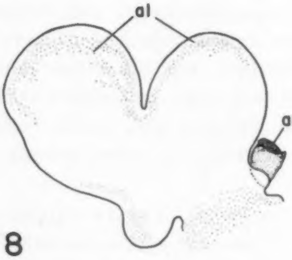
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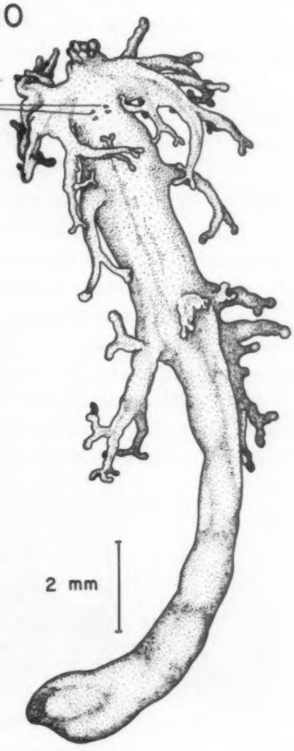
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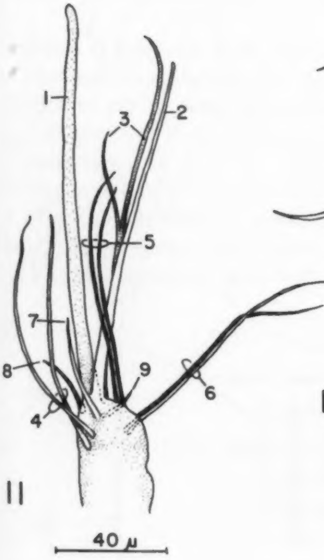
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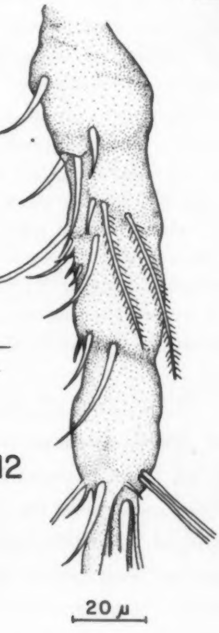
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The second maxilla (Fig. 17) is attached to the inner aspect of the peduncular base of the posterior lobe (*mx″*, Fig. 7). Disposed between closely adhering posterior lobes and the medial lobe, these appendages are orientated in the dorso-ventral plane, rather than crossing the ventral surface and meeting each other in the mid-ventral line as is more common for the lernaeocerids. The second maxilla consists of three segments. Its basal segment constitutes about a half of its length and is provided with two uncinate processes, one ventral and one lateral. The most characteristic feature of these limbs is provided by the middle segment. In addition to the usual pad, subdivided into two and covered with very fine hairs, this segment is also distinguished by the presence, near its distal margin, of a group of fairly long hairs of unequal length, visible at the base of the convex margin of the terminal segment. Another important character is the flagellate seta, present at the base of the opposite margin and extending almost the entire length of the claw. Some specimens appeared to have a second, finer, and shorter seta near the base of the first, but the author could not be quite certain of this. The terminal segment is a claw, constricted at the tip to form a small, nipple-like protrusion. The author was also unable to see whether the claw had the three rows of hairs often found in other genera of Lernaeoceridae (cf. Kabata, 1967a).

Phrixocephalus cincinnatus appears to have only the first two pairs of thoracic legs, which are easily seen, particularly in younger specimens, on the ventral aspect of the cephalothorax, directly below the main trunks of the holdfast (*th*, Fig. 10). The legs consist of apparently one-segmented sympods, bearing two-segmented endopods and exopods.

The first leg (Fig. 19) has the sympod armed with one short, slender, plumose seta on the outer margin, near the base of the exopod. A similar seta is present on the inner margin, at some distance from the base of the endopod. (The plumes are not shown in Fig. 19.) The proximal segment of the exopod has on the outer margin a short, strong spine near the junction with the second segment and a fine, plumose seta in the middle of the inner margin. The margins of the distal segment are armed (counting from lateral to medial side) with a short, robust spine, followed by another spine, about three times as long and with both margins finely serrated. Next follow five long, plumose setae. The

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FIG. 13–19. *Phrixocephalus cincinnatus*, female.

FIG. 13. Second antenna, lateral.

FIG. 14. Intrabuccal stylets and a part of the roof of buccal cavity, lateral.

FIG. 15. Mandible, lateral.

FIG. 16. First maxilla, lateral.

FIG. 17. Second maxilla, lateral.

FIG. 18. Second leg, distal segment of exopod (with part of the first seta enlarged), ventral.

FIG. 19. First leg, entire, ventral. Endopod shown in exploded view; *en*, endopod; *ex*, exopod.

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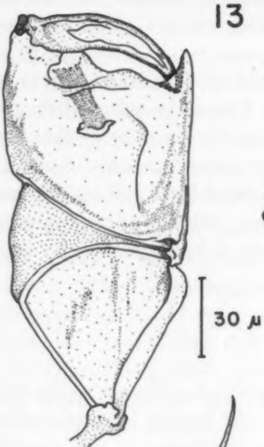

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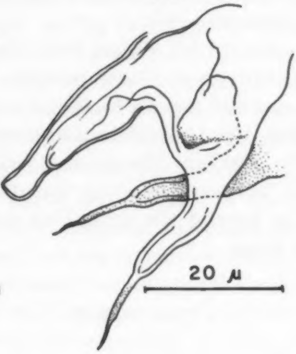

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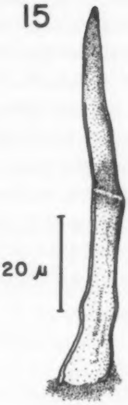

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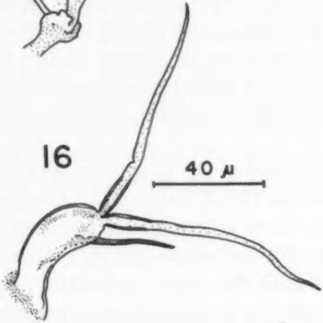

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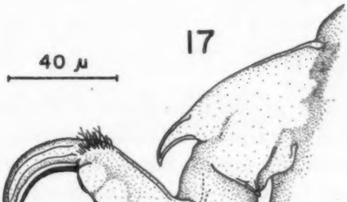

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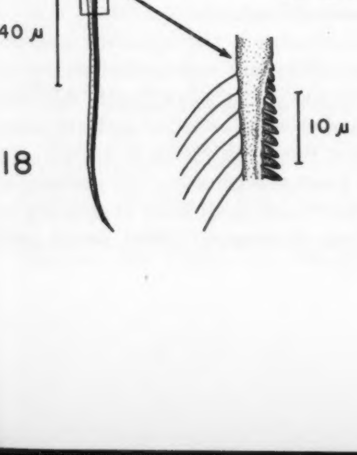

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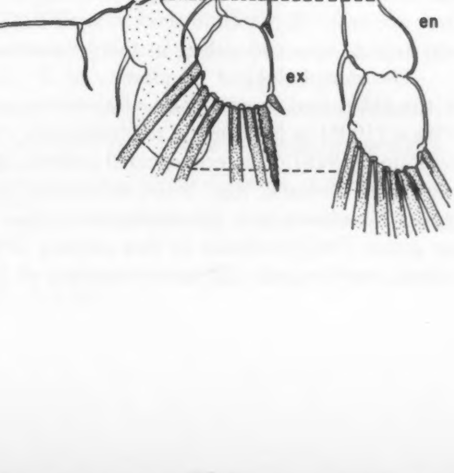

endopod (shown in exploded view in Fig. 19) has its basal segment armed only with one short, plumose seta in the middle of the inner margin. The distal segment bears seven long, plumose setae.

The sympod of the second leg differs from that of the first only in the absence of the plumose seta on its inner margin. The only other difference between the first and the second leg can be found in the armament of the distal segment of the exopod (Fig. 18). It consists of a spine, about twice as long as the corresponding spine of the first leg. The spine is followed by a full-length seta, plumed along one margin but serrated along the other, the serration beginning at some distance from the base of the seta. This seta is followed by the usual set of five long, plumose setae.

DISCUSSION

The examination of the appendages of *P. cincinnatus* has shown again that, in all known instances, the fully modified females of the family Lernaeoceridae possess a complete series of oral appendages (with the exception of the maxillipeds, present only in the males of the family) and both pairs of antennae. This bears out the views expressed by the author on an earlier occasion (Kabata, 1967a).

The previous accounts of *P. cincinnatus* and of its congeners have not included description of their appendages. Wilson (1917) referred to the presence of a chelate second antenna in *P. triangulus* and indicated its position on the dorsal aspect of the cephalothorax, but he did not show any drawings of the appendage itself. Yamaguti (1939) believed that the lobes (= frontal processes of Wilson, 1917, 1922) found on the apex of the cephalothorax represent the first and the second antennae. His view was in agreement with the earlier interpretation of similar lobes in *Lernaeolophus* by Heller (1865) and, later, Wilson (1913). The second maxilla, which he found on the inner aspect of the base of the posterior lobe, was designated by Yamaguti as the endopod of the second antenna. Shiino (1956) pointed out the true nature of this limb. He failed to infer, however, from the relative positions of the maxilla and of the posterior lobe that the latter cannot be an antenna. The present observations should make it clear that the lobes present on the anterior extremity of *P. cincinnatus* do not represent appendages. As in the case of *Lernaeolophus*, they are secondary modifications, with the probable function of protecting the oral appendages and aiding in the procurement of food.

The morphological peculiarity of *P. cincinnatus* is the apparent absence of the third and fourth pairs of thoracopods. This fact was first reported by Wilson (1908) in his original description of the species. It was strongly doubted by Shiino (1956). The lernaeocerid genera usually include species with the same number of thoracic legs, most commonly four pairs. As far as is known, the genera *Peroderma* and *Sarcotretes* have three pairs of legs only. The position of the genus *Phrixocephalus* in this respect is more confused, since it appears to contain species with different numbers of legs. Yamaguti (1939) found only three pairs of legs in *P. cepolae* and, later (1963), on these grounds, made it the type species of *Phrixocephaloides*, although between these two dates Shiino (1956) reported the discovery of the fourth pair. On the other hand, Shiino (1956) described *P. longicollum*, *P. curvatus*, and *P. arboreus*, all with only three pairs of legs. These species were left by Yamaguti (1963) in *Phrixocephalus*, in spite of their obvious failure to conform with his diagnosis of the genus. Moreover, in *P. vipereus* Shiino (1956) has described the fourth pair of legs in vestigial condition, represented by minute papillae, devoid of setae.

These facts raise once again the problem of the taxonomic value of the thoracopods in some parasitic Copepoda, discussed previously by the author (Kabata, 1965). They suggest that the taxonomic value of these appendages has been over-rated and that, although some genera might be characterised by a definite number of legs, this value should more properly be fixed at the specific, rather than the generic, level. Consequently, it seems proper to conclude that there is no basis for the existence of *Phrixocephaloides* Yamaguti, 1963, and that *P. cepolae* should be returned to its original genus, *Phrixocephalus*.

The author took special care in searching for the third and the fourth pairs of legs in *P. cincinnatus*, without success. No traces of them were found either in the fully mature or in the younger females. It is still possible that they were missed in the search and that their number is greater than two pairs. The final answer to this question must await the discovery of an unmodified female or the male of the species.

ACKNOWLEDGMENTS

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REFERENCES

CLEMENS, W. A., AND G. V. WILBY. 1961. Fishes of the Pacific Coast of Canada. 2nd ed. Bull. Fish. Res. Bd. Canada, No. 68. 443 p.

GOODING, R. U., AND A. G. HUMES. 1963. External anatomy of the female *Haemobaphes cyclopterina*, a copepod parasite of marine fishes. J. Parasitol., **49**(4): 663–677.

HELLER, C. 1865. Crustaceen. Reise der Oesterreichischen Fregatte 'Novara' um die Erde, **2**(3): 1–280.

KABATA, Z. 1959 [1960]. *Vanbenedenia krøyeri* (Copepoda parasitica): taxonomic review and other notes. Ann. Mag. Nat. Hist., Ser. 13, **2**: 731–735.

1965. Copepoda parasitic on Australian fishes. III. Genera *Dentigryps*, *Heniochophilus* and *Pseudanuretes* (Caligidae). Ibid., **8**: 19–31.

1967a. The appendages of *Lernaeolophus sultanus* (Milne-Edwards, 1840) (Copepoda: Lernaeoceridae). Crustaceana. (In press.)

1967b. The genus *Haemobaphes* (Copepoda: Lernaeoceridae) in the waters of British Columbia. Canadian J. Zool. (In press.)

SHIINO, S. M. 1956. Copepods parasitic on Japanese fishes. 11. Genus *Phrixocephalus*. Rept. Fac. Fish. Prefect. Univ. Mie, **2**(2): 242–267.

WILSON, C. B. 1908. North American parasitic copepods. A list of those found upon the fishes of the Pacific coast, with descriptions of new genera and species. Proc. U.S. Natl. Mus., **35**: 431–481.

1913. Crustacean parasites of West Indian fishes and crabs. Ibid., **44**: 189–277.

1917. North American parasitic copepods belonging to Lernaeidae with a revision of the entire family. Ibid., **53**: 1–150.

1922. Parasitic copepods from Japan, including five new species. Arkiv Zool., **14**: 1–17.

YAMAGUTI, S. 1939. Parasitic copepods from fishes of Japan. Part 5, Caligoida III. Vol. Jubilare Prof. Yoshida, **2**: 443–487.

1963. Parasitic Copepoda and Branchiura of fishes. Interscience Publishers, New York, London, and Sydney. 1104 p.

Quality of Pacific Coast Dogfish During Frozen Storage[1]

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ABSTRACT

Pacific coast dogfish (*Squalus suckleyi*) were skinned, frozen, glazed, and stored at –5, –10, –20, and –30 C to determine the extent of oxidative rancidity, colour change, protein denaturation, and lipid hydrolysis during storage. Belly flaps from dogfish carcasses were tested under similar conditions. Discoloration of the red outer muscle of carcasses was the initial significant change in quality. Initial odour and flavour changes in defrosted carcasses were detected after 22 and 70 days storage at –10 and –20 C respectively; no significant changes were evident in carcasses after 180 days storage at –30 C. Slight fading of the pink colour of belly flaps was observed after storage for 50 days at –20 C and for 160 days at –30 C. A slight acidic type flavour was detected in belly flaps after 99 days storage at –20 C.

There was a slight relationship between the extent of protein denaturation and lipid hydrolysis in dogfish muscle during frozen storage.

INTRODUCTION

Pacific coast dogfish *Squalus suckleyi* (Girard), members of the shark family, are a problem to commercial and sport fishermen. Economic losses to commercial fishermen, resulting from the presence of an excessive number of dogfish in fishing areas, are brought about by damage to gear and loss of fishing time. During 1965 the Department of Fisheries of Canada initiated programs in an attempt to assist in controlling the dogfish population in the offshore waters of British Columbia. One of the main programs was an experiment concerned with the marketing of dogfish carcasses, belly flaps, and processed products for use as human food. Tarr (1958) discussed utilization of dogfish; he suggested that a profitable use of dogfish might be possible if an export market could be found for them as an edible fish. Alverson and Stansby (1963) reported on three general methods for use in the control of dogfish populations; one method which they suggested was the use of dogfish as food for human consumption.

Although reports have been published concerning the quality of nonfrozen dogfish during storage (Moyer et al., 1959; Southcott et al., 1960), a survey of the literature revealed that there was a lack of information concerning development of oxidative rancidity and colour changes in frozen dogfish during storage. In conjunction with the experimental dogfish marketing program initiated by the Department of Fisheries, an experiment was carried out to assess the stability of dogfish muscle during frozen storage for 4–5 months.

This paper presents information concerning rancidity development, colour changes, protein denaturation, and lipid hydrolysis in frozen dogfish.

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MATERIALS AND METHODS

Fresh dogfish (24 hr post-mortem), which were caught during the month of January, were eviscerated, skinned, beheaded, frozen, and glazed at a commercial fish processing plant. Belly flaps (BF) cut from dogfish carcasses were packed and frozen in 5-lb cartons. Approximately 200 lb of frozen carcasses and 40 lb of frozen BF were obtained for storage tests. Carcasses, which were packed in polyethylene bags, and BF in cartons were stored under laboratory conditions at −10, −20, and −30 C. For extreme accelerated effects of storage, frozen carcasses were stored at −5 C. At intervals samples were removed from storage to ascertain the effect of frozen storage on quality.

Oxidative rancidity in frozen muscle was determined by organoleptic evaluation of thawed samples and by determination of the peroxide value (PV) of extracted lipids as measured by the method of Tarr (1947). In this method, the PV is calculated as the number of milliliters of 0.002 N sodium thiosulfate solution required in the titration of 1 g of lipid. Peroxide values were determined on separate composite samples of homogenized white inner and red outer muscle obtained from head, body, and tail sections of at least two carcasses. The outer layer of red muscle which was sliced from carcasses was approximately 5 mm thick. To ascertain rancidity development in BF, three were used for each determination. Samples were homogenized for 1 min in a Servall omni-mixer in preparation for lipid extraction.

To prepare cooked samples for organoleptic evaluation, freshly defrosted portions of dogfish were placed in petri plates and cooked for 10 min at 10 lb pressure (115 C).

Protein denaturation was determined by extracting protein soluble in a solution of 5% NaCl in 0.02 M $NaHCO_3$ (Dyer et al., 1950); the method was slightly modified by Tomlinson and Geiger (1963). Total protein nitrogen in frozen dogfish muscle was determined by the method of Torten and Whitaker (1964). The heating time of 10 min used in the extraction was reduced to 5 min as suggested by N. Tomlinson and S. E. Geiger (unpublished data).

Total lipid in dogfish muscle and BF was determined by the method of Bligh and Dyer (1959). Free fatty acid (FFA) formation from the hydrolysis of lipid during storage was determined by the method of Dyer and Morton (1956).

Measurements of *p*H were made on portions of whole muscle or on homogenized muscle using a Siebold Model GLD *p*H meter. For the determination of muscle water content, portions of muscle were homogenized in a Servall omnimixer, and the per cent moisture of samples determined on a direct-reading moisture balance.

Colour changes were assessed by visual examination of the outer surface of skinned carcasses and BF under an incandescent lamp. A differential colour instrument (Model C4 Gardner Colour Difference Meter) was also used to make colour measurements on samples from unglazed carcasses after 75 days storage and from glazed carcasses and BF after 115 days storage. Samples were prepared for instrumental colour measurements by removing a 5-mm layer of outer muscle from the head, body, and tail sections of 2–3 skinned carcasses. Cut portions of each sample were homogenized for 1 min at 0 C with a Servall omni-mixer; the homogenate was placed in a clear plastic container, and measurements made within 4 min. Cut portions of 3 BF from each storage temperature were homogenized and treated similarly. The results were calculated in terms of ΔE (Judd, 1952), the total colour difference in NBS (National Bureau of Standards) units between a sample and a fresh sample.

RESULTS

In Table I, the results of storage tests on frozen carcasses indicate that dogfish lipids undergo oxidation. Changes in colour from the normal red-pink of skinned dogfish occur before extreme organoleptic changes are evident; at −10 and −20 C colour fading and yellowing were evident before any other significant changes were noted. Differences in colour of the outer surfaces of carcasses after 36 days storage at −5, −20, and −30 C are illustrated in the accompanying photograph (Fig. 1). The red colour of the −30 C carcass is evident whereas fading is noticeable in the other samples. Initial fading of colour was evident after storage for 12, 19, and 70 days at −10, −20, and −30 C respectively. Initial odour and flavour changes were noted after storage for 22 days at −10 C, and 70 days at −20 C; at −30 C no significant changes were evident after 180 days storage.

The comparative results (Table II) of visual observations and colour measurements on glazed and unglazed carcasses and BF indicate that an appreciable colour change does occur during storage and that there is an association between the visual judgments and the ΔE values. Colour changes were greater at the higher storage temperatures. The ΔE values of BF samples after 115 days, storage at −10 and −20 C were 4.3 and 2.7 respectively when compared to the samples stored at −30 C. The ΔE values of samples from glazed carcasses after 115 days storage did not show as great a variation with respect to storage temperatures as did those for unglazed carcasses, due to protection provided by the glaze. Samples from unglazed carcasses that had been stored at −10, −20, and −30 C for 75 days had ΔE values of 9.5, 5.0, and 2.6 respectively.

Ammoniacal odours were not detected at any time during examination of defrosted carcasses; however, an ammoniacal type off-odour was noticed from some carcasses in which the kidney tissue had not been removed during processing. E. M. Raynor (personal communication) reported that no organoleptic changes were evident in cooked samples of commercially processed dogfish carcasses after 3 months storage at −22 C under commercial conditions.

The average moisture content of dogfish muscle was 70%. The *p*H of fresh dogfish muscle was 6.15; after 115 days storage at −10, −20, or −30 C the *p*H was 6.1.

In one separate trial the PV of extracted lipid of unglazed dogfish carcasses stored for 90 days at −10, −20, and −30 C were 22, 6.4, and 1.3 respectively. In another trial the pretreatment of dogfish portions with a 1% ascorbic acid

TABLE I. Peroxide values and organoleptic evaluation of frozen glazed dogfish during storage at –5, –10, –20, and –30 C.

Storage		PV[a]		Colour[b]		Odour		
Temp(*C*)	Days	Inner	Outer	Uncooked	Cooked	Uncooked	Cooked	Flavour
–5	7	0	1.1	4		5		
	26	0	5.6	4	Brown	6	7	4
	36	15.3	30.4	2			2	2
–10	7	0	0.1	8				
	26	0.8	2.2	5	Light grey	9	9	8
	36	1.9	2.0	4			8	6
	70	6.5	5.4	5	Brown		6	4
	115		20.0	4		5	4	3
–20	7	0	0.3	8		9	9	9
	26	0.7	0.3	6	Light grey	9	9	9
	36	1.2	2.5	6				
	70	0.8	0.6	5	Grey; brown	8	8	9
	115		1.8	4	Grey; brown	8	7	8
	180		4.7	3	Brown	5	5	4
–30	7	0	0	10				
	26	0	0	10	Light grey	10	10	10
	36	0.9	0.6	9				
	70	1.3	1.2	8	Light grey; brown	9	9	10
	115		0.8	7	Light grey; brown	9	9	10
	180		1.4	5	Grey; brown	9	9	9

[a] Peroxide values were determined on white inner muscle and on a 5-mm layer of red outer muscle.

[b] Colour changes were assessed by visual examination of red outer muscle. Colour code: 10-8, pink; 7-5, faded pink; 4-1, light yellow and brown. Odour and flavour code: 10, sweet; 9, not rancid; 8-6, off odour, flavour; 5, slightly rancid; 4-1, rancid.

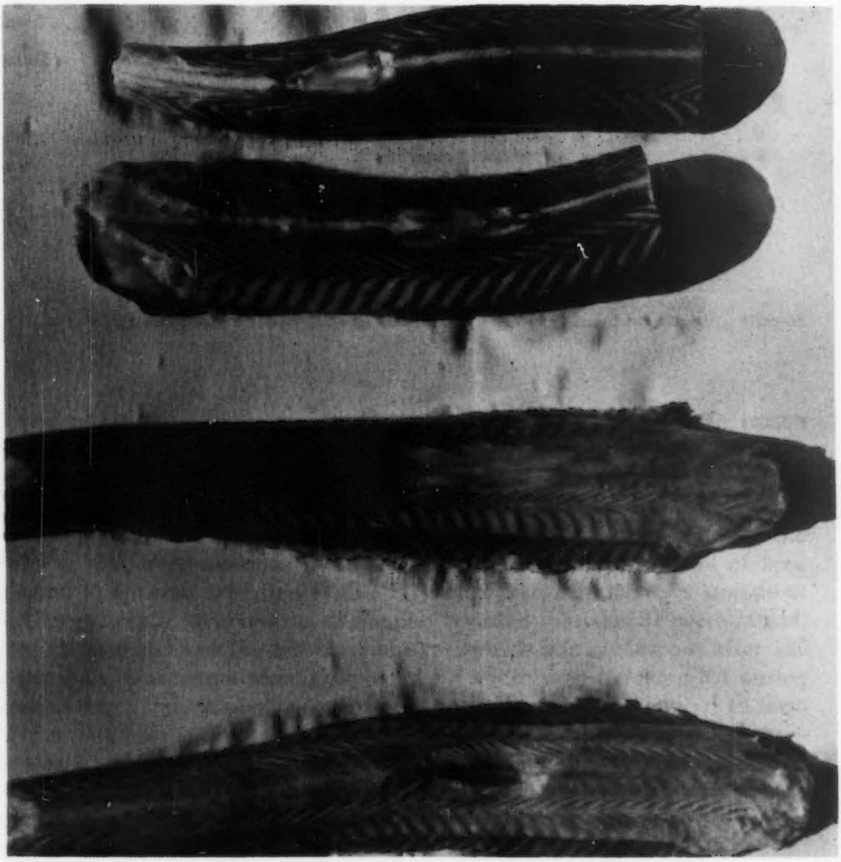

FIG. 1. Change in colour of skinned frozen dogfish carcasses after 36 days storage at −30 C, upper fish in photograph; −20 C, center fish; −5 C, lower fish.

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TABLE II. Effect of storage on colour changes in frozen dogfish.

Sample	Temp (*C*)	Days	Visual appearance	ΔE
Fresh	–	0	Red, pink	0
Commercial, glazed	–24	115	Slight pink with fading & browning	6.6
Unglazed	–10	75	Faded pink, yellowing, brown	9.3
"	–20	"	Faded pink, faded brown	5.0
"	–30	"	Pink	2.6
Glazed	–10	115	Slight pink, faded brown & yellow	7.9
"	–20	"	Slight pink, faded brown & yellow	6.3
"	–30	"	Faded pink, faded brown	6.1
Belly flaps	–10	"	Faded pink, yellowing, white	4.3[a]
" "	–20	"	Faded pink, slight yellowing	2.7[a]
" "	–30	"	Pink, slight fading	0

[a] Total colour difference in NBS units as compared to samples stored for the same time at –30 C.

dip was effective in retarding development of oxidative rancidity in frozen dogfish muscle; after 79 days storage at –10 C the PV of treated and untreated portions were 0.4 and 4.8 respectively. However, pigments of the red muscle were altered by the ascorbic acid treatment.

Although the average lipid content of BF was 18.9 g/100 g muscle, the results of PV determinations indicate that only a small percentage of lipid appeared to undergo oxidation (Table III). However, after 36 days storage at –10 C organoleptic examination of samples revealed that the BF were rancid. Table III also includes the results of one test which was carried out after 130 days storage to ascertain whether lipids of BF hydrolyzed to form FFA during frozen storage. Only a small percentage of total lipid was hydrolyzed to form FFA; at –10, –20, and –30 C the FFA (as oleic acid) formed from lipid hydrolysis was 2.5, 1.18, and 0.51% respectively of extracted lipid. Dyer and Fraser (1959) reported that FFA development is slower in fish of high fat content than in fish of low fat content, but that FFA development is still temperature dependent.

The results of protein denaturation and lipid hydrolysis tests on frozen dogfish carcasses indicate that protein denaturation and lipid hydrolysis do occur in frozen dogfish muscle during storage (Table IV). After 135 days storage at –10 C the amount of total protein soluble in high ionic-strength salt solution (Dyer et al., 1950) was 57%; at –30 C storage 92% was soluble. FFA development resulting from the hydrolysis of lipid in muscle of carcasses was slightly greater than that reported for BF (Table III), although similar with regard to temperature effect and rate of development.

TABLE III. Peroxide values, organoleptic evaluation, total lipid and free fatty acid content of frozen dogfish belly flaps stored at –10, –20, and –30 C.

Storage			Colour[a]		Odour			Lipid	
Temp(C)	Days	PV	Uncooked	Cooked	Uncooked	Cooked	Flavour	Total %	Hydrolyzed % FFA[b]
–10	36	4.6	6		5				
	70	5.9	5		5				
	99	5.0	5		4				
	130	4.3	4	Browning	3	4	4	17.0	2.50
	160	8.2	3	Brown		2	3		
–20	36	0.5	8		9				
	70	1.1	7		9				
	99	2.4	7		7				
	130	1.7	6	White, slight yellowing	8	8	7	19.8	1.18
	160	1.7	5	Grey, white	7	7	6		
–30	36	0.8	10		9				
	70	1.3	10		9				
	99	0.6	9		9				
	130	0.8	9	White	9	9	10	20.0	0.51
	160	1.0	8	Grey, white	9	10	10		

[a] Colour code: 10-8, pink; 7, faded pink; 6-5, faded pink, light yellow; 4-1, light yellow and brown. Odour and flavour code: 10, sweet; 9, not rancid; 8-6, off odour, flavour; 5, slightly rancid; 4-1, rancid.

[b] Free fatty acid content (as oleic acid) of extracted lipid.

TABLE IV. Soluble protein nitrogen, total lipid and free fatty acid content of frozen dogfish muscle.

Storage		Protein N			Lipid	
Days	Temp(*C*)	Total (*mg/g muscle*)	Soluble[a] (*mg/g muscle*)	% soluble	Total %	Hydrolyzed % FFA[b]
30	−5	22.7	14.9	66.		
30	−30				14.0	0.9
120	−5				13.7	3.3
"	−10	24.5	16.5	67.	11.4	4.6
"	−20	24.2	22.0	91.	14.4	1.4
"	−30	23.5	21.0	89.	15.2	1.3
135	−10	21.5	12.2	57.		
135	−30	23.2	21.5	92.		

[a] Soluble in 5% NaCl, 0.02 M $NaHCO_3$ solution.
[b] Free fatty acid content (as oleic acid) of extracted lipid.

DISCUSSION

The results of this investigation indicate that discoloration of red outer muscle of skinned frozen dogfish during storage is the initial noticeable deterioration in quality. However, objective and subjective tests on dogfish during the initial stage of discoloration revealed that muscle lipids had not undergone oxidation or hydrolysis. The discoloration of red muscle of dogfish during storage is thought to be due to the oxidation of hemoglobin to form methemoglobin. The next changes which were evident after the initial discoloration were development of an acidic type flavour and rancidity. The rate of development of oxidative rancidity (as determined by PV) in frozen dogfish muscle stored at −10 C (Table I) was compared with the reported rate of rancidity development in species of salmon and sablefish (*Anoplopoma fimbria*) during storage at −10 C (Tarr, 1946 and 1947). Tarr reported the following PV: 3.4 and 10.1 for red chinook salmon (*Oncorhynchus tshawytscha*) after 70 and 120 days storage respectively; 20 for chum salmon (*Oncorhynchus keta*) after 103 days storage; and 2.1 for sablefish after 69 days storage. Although methods of handling, glazing, and storing fish samples in the separate experiments would not be comparable in some respects, the comparisons indicate that lipids of frozen dogfish undergo oxidation at a similar or greater rate than do lipids of certain other species of fish.

The results of protein denaturation and lipid hydrolysis tests on frozen dogfish muscle (Table IV) indicate a relationship between these two biochemical changes during frozen fish storage as suggested by Dyer and Fraser (1959) and Olley and Lovern (1960). After 120 days storage of carcasses at −10 C the FFA formed was 4.6% of the total lipid extracted; on a wet muscle basis this was equivalent to 524 mg FFA/100 g muscle. The soluble protein nitrogen content was 67 and 57% after 120 and 135 days respectively. The results are in accordance with those reported by Olley et al. (1962); after 20 weeks, storage at –14 C they found the FFA content of extracted lipid from dogfish muscle was approximately 5%; on a wet muscle basis they reported the FFA content was approximately 500 mg FFA/100 g muscle. They reported that the protein nitrogen soluble in a 5% NaCl solution after 18–20 weeks storage at –14 C was 52–59%. In the present work the average total lipid content of dogfish muscle was 13.7% which was in agreement with the 14% lipid content reported by Olley et al. (1962) for dogfish caught in February. They also reported that the *p*H of frozen dogfish muscle did not change after 70 weeks storage at –14 C from an initial *p*H of 6.1 for fresh dogfish; our results concerning *p*H were in accordance with these findings. Olley et al. (1962) suggested that a lack of change in *p*H during storage might be due to the buffer capacity of proteins, soluble salts, and alkaline phosphates which might be different in dogfish muscle than in other species of fish.

The results as presented in this paper indicate that dogfish muscle is susceptible to lipid oxidation and hydrolysis, protein denaturation, and colour change during frozen storage. It might be assumed that under commercial conditions of handling and storage, the quality of frozen dogfish carcasses and belly flaps could possibly be maintained during storage at –20 to –25 C for 3–5 months. If a market becomes available for dogfish carcasses, it is suggested that kidney tissue in the dorsal region of the peritoneal cavity should be removed during processing in an attempt to prevent an unnecessarily early development of off-odours. A glaze applied to frozen dogfish carcasses would assist in retarding lipid oxidation and colour change during storage.

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REFERENCES

Alverson, D. L., and M. E. Stansby. 1963. The spiny dogfish (*Squalus acanthias*) in the northeastern Pacific. U.S. Fish Wildlife Serv., Spec. Sci. Rept., Fish. No. 447. p. 1–25.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. Physiol., **37**: 911–917.

Dyer, W. J., and D. I. Fraser. 1959. Proteins in fish muscle. 13. Lipid hydrolysis. J. Fish. Res. Bd. Canada, **16**: 43–52.

Dyer, W. J., H. V. French, and J. M. Snow. 1950. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. Ibid., **7**: 585–593.

Dyer, W. J., and M. L. Morton. 1956. Storage of frozen plaice fillets. Ibid., **13**: 129–134.

JUDD, D. B. 1952. Color in business, science and industry. John Wiley and Sons, New York, N.Y. 401 p.

MOYER, R. H., B. A. SOUTHCOTT, E. G. BAKER, AND H. L. A. TARR. 1959. Keeping quality of Pacific coast dogfish. J. Fish. Res. Bd. Canada, **16**: 791–794.

OLLEY, J., AND J. A. LOVERN. 1960. Phospholipid hydrolysis in cod flesh stored at various temperatures. J. Sci. Food Agr., **11**: 644–652.

OLLEY, J., R. PIRIE, AND H. WATSON. 1962. Lipase and phospholipase activity in fish skeletal muscle and its relationship to protein denaturation. Ibid., **13**: 501–516.

SOUTHCOTT, B. A., R. H. MOYER, E. G. BAKER, AND H. L. A. TARR. 1960. Keeping quality of Pacific coast dogfish II. J. Fish. Res. Bd. Canada, **17**: 811–814.

TARR, H. L. A. 1946. Control of rancidity in stored fish. III. Progr. Rept. Pacific Coast Sta., Fish. Res. Bd. Canada, No. 68. p. 13–16.

1947. Control of rancidity in fish flesh. 1. Chemical antioxidants. J. Fish. Res. Bd. Canada, **7**: 137–154.

1958. Dogfish. Prog. Rept. Pacific Coast Sta., Fish. Res. Bd. Canada, No. 110. p. 13–16.

TOMLINSON, N., AND S. E. GEIGER. 1963. The bound nucleotides of freshly frozen and severely denatured frozen lingcod muscle. J. Fish. Res. Bd. Canada, **20**: 187–194.

TORTEN, J., AND J. R. WHITAKER. 1964. Evaluation of the biuret and dyebinding methods for protein determination in meats. J. Food Sci., **29**: 168–174.

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The Significance of Ventral Bioluminescence in Fishes[1]

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ABSTRACT

The positions of luminous organs and tissues are documented for all known bioluminescent families of fishes. The majority of families, species, and individuals possess photophores only or mainly on the ventral half (as opposed to the dorsal half) of the body. The position and in some cases also the photophore structure tend to direct the emitted light downwards. Review of the previously suggested functions of luminescence and a new one (proposing light organs may function in the spacing out of midwater fish in the absence of marks for fixing territories) suggests that these functions are not related to the ventral predominance of luminescence. Photophores functioning as lures for prey and as headlights do not appear to follow the general tendency for ventral positioning of bioluminescence.

Study of the two previously proposed theories on ventral predominance of luminescence, the reflector theory and the "counter-shading" or disruptive illumination theory, shows that there are some cases which these theories do not explain; these two theories apply only during the day. A third theory is developed which suggests that the larger numbers of predators in upper waters would select for fishes with ventrally directed nocturnal luminescence as this would subject them to less predation. Fishes *possess* photophores for a number of reasons, but they are *placed* ventrally because this results in decreased predation. Some counter-strategies are discussed, showing how fishes have coped with certain problems created by bioluminescence.

RÉSUMÉ

On confirme la position des organes et du tissu lumineux dans toutes les familles connues de poissons luminescents. La majorité des familles, des espèces et des individus possèdent des photophores uniquement ou principalement sur la moitié ventrale de leur corps (par opposition à la moitié dorsale). La position et parfois la structure des photophores ont tous deux tendance à diriger la luminescence émise vers le bas. Lorsqu'on examine les fonctions de luminescence avancées auparavant, ainsi qu'une nouvelle (à supposer que les organes lumineux servent à éloigner les poissons des eaux médianes, en l'absence de marques pour déterminer les espaces), il appert que ces fonctions ne sont pas apparentées à la prédominance ventrale de la luminescence.

Une étude des deux théories proposées plus tôt relativement à cette prédominance ventrale de la luminescence, la théorie du réflecteur et celle de l'illumination faisant opposition à l'ombre ou de l'illumination interrompue, montre que ces théories n'expliquent pas tout, et elles ne s'appliquent que pendant le jour. On développe une troisième théorie qui veut qu'un grand nombre de prédateurs nageant près de la surface sélectionnant les poissons émettant une luminescence nocturne du côté ventral qui les exposerait moins à la rapacité. On parle de tactiques destinées à faire échec aux premières, de la façon dont les poissons ont réglé certains problèmes causés par la bioluminescence.

INTRODUCTION

Bioluminescence or living light is found in many taxa of fishes and other animals and has probably evolved independently several times even in fishes. A striking feature is the predominantly ventral situation of the luminescence.

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Most photophores or luminous tissues in most fishes are found on the lower half of the fish. That this tendency is exhibited in several taxa of fishes as well as in squids suggests that there is a strong selective factor or factors producing this uniformity in position. This paper tries to explain what selective factors are involved. A summary of fish taxa known to be luminous is provided.

The phenomenon of bioluminescence has been surveyed several times. Harvey (1952) devotes a volume to the occurrence, structure, physiology, and function of light organs and tissues. Marshall (1954) presents a chapter on 'living light.' Harvey (1957) and Nicol (1960) also give a chapter on luminescence. These provide sources of information and literature in this field. The more recent and pertinent literature will be referred to further on in this paper. (See Zahl (1953) for coloured photos of live specimens.)

The terms *photophore* and *luminous organ* or *light organ* are used for light-emitting bodies with constant form or structure, often backed by black absorbing tissue and provided with a lens and a reflector. The term *luminous tissue* or *gland* is used when constant form and light-focusing structures are lacking (see discussion by Parr, 1960, p. 8 and Fraser-Brunner, 1949, p. 1037).

PHOTOPHORE POSITION

The predominance of photophores on the ventral side of fishes and other animals has been noted by Walls (1942), Marshall (1954), Harvey (1957), and Clarke (1963). Harvey (1957) states, "photophores occur in every conceivable position, although they are usually ventral or lateral rather than dorsal." This view is documented in Table I. Figures 1 and 2 show the patterns of luminescence in the major groups of bioluminescent fishes.

Table I gives the locations of photophores and luminous tissue of all families of fishes known to exhibit bioluminescence. The taxa are listed in phylogenetic sequence with the more primitive forms near the beginning. Classification for the bony fishes follows McAllister (1967). In Table I, dorsal and ventral mean above and below a median horizontal plane dividing the body in two equal halves. Organs which cross the plane are described as mid-lateral.

Questionably luminous forms are not included in the table (see Harvey, 1957, when no other author indicated). These forms include the halosaurid genera *Aldrovandia* and *Halosaurus*, the brotulid genera *Bassocetus*, *Leucicorus*, and *Lamprogrammus*, the macrourid genus *Macrourus*, the bathylaconid genus *Bathylaco* (the club-shaped structures above the gill opening), the kasidorid genus *Kasidoron* (Robins and de Sylva, 1965), the searsid genera *Maulisia* and *Normanichthys* (dermal pits) and *Barbantus* (mid-ventral strip, Parr, 1960), the gempylid genus *Ruvettus*, and the trachichthyid genus *Hoplostethus* (Cohen, 1964a). Further, Haneda (1950) has shown that *Harpodon* (Harpodontidae) is not luminous when alive and Munk (1959) has demonstrated that the orbital organs of *Ipnops* (Ipnopidae) are modified eyes rather than luminous organs as has been suggested by some authors. It might also be mentioned that *Somniosus microcephalus* (Squalidae) has been found frequently to have luminescent parasites on its eyes in Greenland waters.

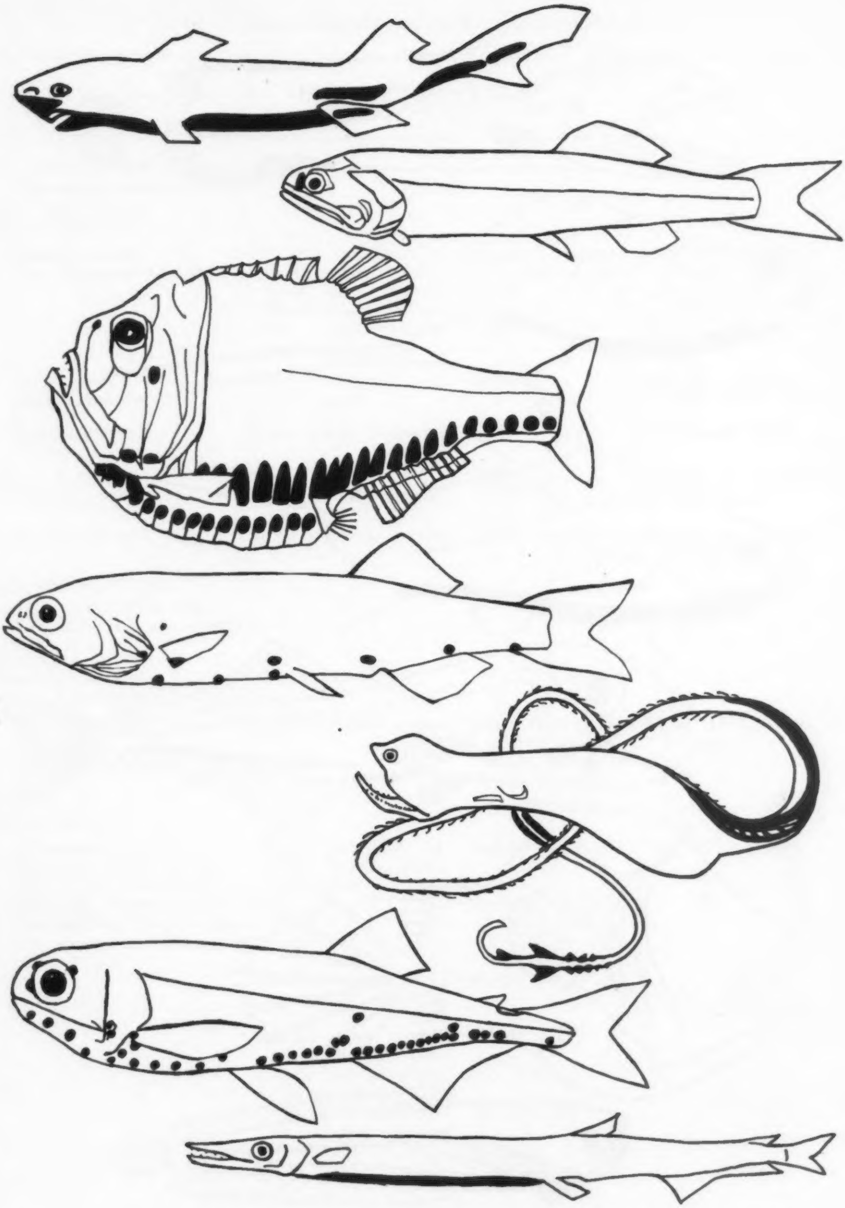

FIG. 1. Distribution of bioluminescence in examples of major luminescent groups. Top to bottom: Squalidae, Bathylaconidae, Sternoptychidae, Searsidae, Saccopharyngidae, Myctophidae, and Paralepidae.

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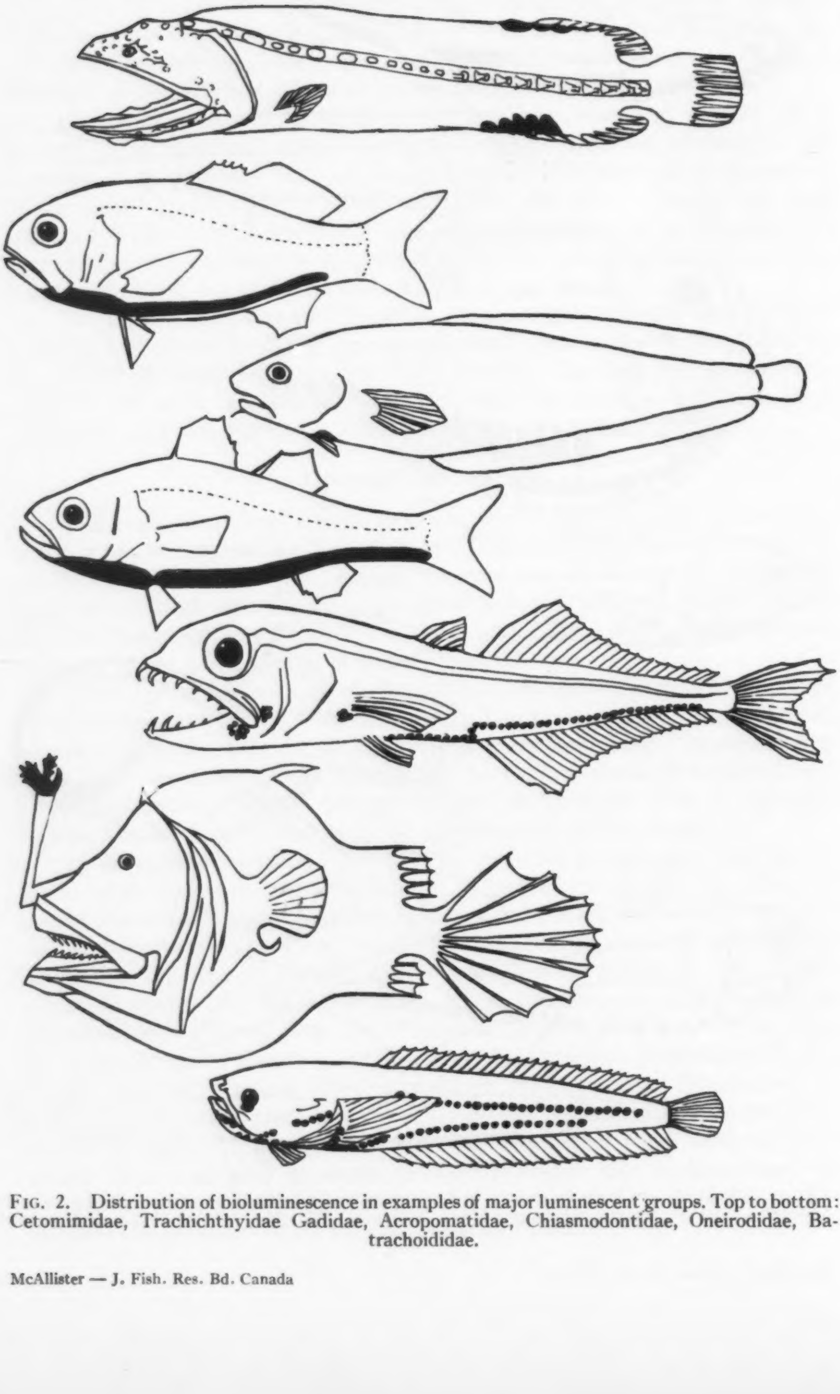

FIG. 2. Distribution of bioluminescence in examples of major luminescent groups. Top to bottom: Cetomimidae, Trachichthyidae Gadidae, Acropomatidae, Chiasmodontidae, Oneirodidae, Batrachoididae.

TABLE I. Photophore positions in bioluminescent fishes.

Taxa	Luminous tissue or organ	Position	Source
Class ELASMOBRANCHOMORPHI			
Subclass ELASMOBRANCHII			
Order Squaliformes			
Family Squalidae			
Centroscyllium (incl. *Paracentroscyllium*)	Tip and underside of head, upper eyelid, base of pectoral, flank, caudal peduncle (luminescence sparsest dorsally)	Mainly ventral, ventral, or all over	Iwai (1960b); Burckhardt (1900); Parin (1964)
Etmopterus (incl. *Spinax*)	Whole underside and near mouth	Ventral	Harvey (1957); Parin (1964)
Somniosus[a] (incl. *Laemargus*)	Head, back, and tail	Dorsal	Burckhardt (1900)
Family Dalatiidae			
Isistius	Entire lower trunk, paired and caudal fins	Mainly ventral	Burckhardt (1900); Parin (1964)
Euprotomicrus	Head and body organs	Mainly ventral	Burckhardt (1900); Parin (1964)
Class TELEOSTOMI			
Subclass ACTINOPTERYGII, TELEOSTEI, MALACOPTERYGII			
Order Clupeiformes			
Suborder Clupeoidi			
Family Alepocephalidae			
Xenodermichthys, *Photostylus*, *Anomalopterichthys*	Body and head organs	Usually mainly ventral, sometimes about equal dorsally and ventrally	Misra (1953); Beebe (1933a)
Family Searsidae			
Mirorictus, *Searsia*, *Sagamichthys*, *Mentodus*, *Persparsia*, *Holtbyrnia*, *Maulisia*, *Normanichthys* (all head and body organs not present on all genera)	Head organs (GO, OO, IOO, BRO, POO, and OPO)	Mainly ventral	Parr (1951, 1960)
	Body organs (JO, PAO, ICO, CO, IPO, PO, THO, PVO, SVO, AO, SAO)	Ventral except SPO is dorsal and CO is midlateral	
	Shoulder organ (all 12 genera of the family)	Dorsal	
	Dermal pits (?luminous): (a) large cleithral ones (*Maulisia*, *Normanichthys* only); (b) minute ones on most scales	Dorsal All over body	
	Luminous tissue	Ventral (except supracaudal in *Plathytroctes*)	

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[a]See footnote bottom of concluded Table.

(*Continued*)

TABLE I. Photophore positions in bioluminescent fishes. — (*Continued*)

Taxa	Luminous tissue or organ	Position	Source
Suborder Stomiatoidei			
Families Gonostomatidae, Sternoptychidae, Stomiatidae, Chauliodontidae, Astronesthidae, Malacosteidae, Melanostomiatidae, Indiacanthidae (see Harvey 1952, for luminous genera (54 in 8 families))	Body organs Head organs, including barbel and teeth	Ventral (except all over in a few like *Malacosteus indicus* and *Bathophilus brevis*) Mainly ventral	Harvey (1952); Marshall (1954); Misra (1953); Beebe and Crane (1939)
Suborder Bathylaconoidei			
Family Bathylaconidae			
Bathylaco	Preorbital organ	Dorsal	Bigelow (1964)
Suborder Salmonoidei			
Family Bathylagidae			
Bathylagus	Abdominal scales, anal and caudal bands	Ventral	Beebe (1933b and c)
Family Opisthoproctidae			
Opisthoproctus and *Winteria*	Rectal bulb reflecting ventrally	Ventral	Bertelsen and Munk (1964); Cohen (1964b)
Dolichopteryx	Orbital photophore Abdominal midventral organs Middorsal organs	Dorsal Ventral Dorsal	
Rhynchohyalus	Suborbital organ Rectal gland	Midlateral Ventral	
Bathylychnops	Suborbital organ Orbital tissue	Ventral Dorsal	
Order Saccopharyngiformes			
Family Saccopcaryngidae			
Saccopharynx	Nuchal troughs Caudal organs	Dorsal Dorsal and ventral	Beebe (1932)
Family Eurypharyngidae			
Eurypharynx	Dorsal troughs Caudal organs	Dorsal Dorsal and ventral	Bertin (1934)
TELEOSTEI, ACANTHOPTERYGII			
Order Myctophiformes			
Suborder Myctophoidei			
Family Myctophidae (30 genera)	Body photophores	Ventral or mainly ventral (except 2 genera); usually all ventral, but often the PLO, sometimes the VLO, SAO,	Fraser-Brunner 1949; Bolin (1961, 1966)

(*Continued*)

Table I. Photophore positions in bioluminescent fishes. — (*Continued*)

Taxa	Luminous tissue or organ	Position	Source
		POL, and Prc may be dorsal (e.g. *Notolynchus*) but even when these are dorsal the majority of organs are ventral except in *Scopelopsis* which has one on every scale and some *Lampanyctus* which have secondary ones under every scale but retain the large ventral ones	
	Caudal glands ('stern chasers')	Dorsal and ventral; in some sp. dorsal only in the ♂ and ventral only in ♀, e.g. *Myctophum* sp.; in others dorsal and ventral in both sexes, e.g. *Lampadena* sp., or in the ♂ only, e.g. *Tarletobeania taylori;* in a few sp. dorsal only, and only in the ♂, e.g. *Loweina* sp. and *Tarletobeania crenularis*	
	Glands on bases of dorsal, pectoral, pelvic, anal, thoracic, pre- and post-dorsal, and above anterior lat. line (only in a few species)	Dorsal and ventral	
	Head photophores (Br, So, Bu, Op, Vn, Dn, Ce)	Ventral (except Dn and Ce)	
	Head glands (rare)	Dorsal	
Family Neoscopelidae			
Neoscopelus	Body photophores Tongue	Ventral Ventral	Fraser-Brunner (1949); Harvey (1952)

(*Continued*)

TABLE I. Photophore positions in bioluminescent fishes. — (*Continued*)

Taxa	Luminous tissue or organ	Position	Source
Suborder Alepisauroidei Family Paralepididae			
Lestidium and *Lestrolepis*	Abdominal organ	Ventral	Haneda (1958); Rofen (1966a)
Order Cetomimiformes Family Cetomimidae	Cavernous ridged luminous tissue:		Harrey (1952)
	(a) near anus (usual in *Ditropichthys*, *Cetomimus* and *Cetostoma*);	Ventral	
	(b) anal fin (usually in *Cetomimus*);	Ventral	
	(c) dorsal fin (often in *Cetomimus*)	Dorsal	
Order Beryciformes Suborder Berycoidei Family Trachichthyidae			
Paratrachichthys	Internal anal gland with ventral body wall translucent	Ventral	Haneda and Johnson (1962); Harvey (1957)
Family Anomalopidae			
Anomalops and *Photoblepharon*	Suborbital organ	Midlateral	Harvey (1957)
Family Monocentridae			
Monocentris	Submandibular organ	Ventral	Harvey (1957)
Order Gadiformes Suborder Gadoidei Family Gadidae			
Physiculus and *Lotella*	Anal gland	Ventral	Harvey (1957); Cohen (1964a)
Steindachneria	Anal gland, lower body and head, and opercular region	Mainly ventral	
Suborder Macrouroidei Family Macrouridae			
Malacocephalus, *Grenurus*, *Hymenocephalus*, *Coryphaenoides* (incl. *Nezumia*), *Trachoneurus*, *Coelorinchus*, *Ventrifossa*	Anal gland	Ventral	Harvey (1957); Haneda (1951)
Order Perciformes Suborder Percoidei Family Acropomatidae			
Acropoma	Thoracic gland, long duct ending near anus, ventral body wall translucent	Ventral	Haneda and Johnson (1962)
Family Apogonidae[b]			
Apogon	Anal and thoracic glands, intestinal diverticula, translucent thorax;	Ventral	Haneda and Johnson (1962)

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[b] See footnote bottom of concluded Table.

(*Continued*)

TABLE I. Photophore positions in bioluminescent fishes. — (*Concluded*)

Taxa	Luminous tissue or organ	Position	Source
Siphamia	Thoracic gland with duct to intestine, translucent venter	Ventral	Iwai (1958; 1960a)
Family Leiognathidae			
Gazza, *Leiognathus* (incl. *Secutor*)	Circumesophageal gland at stomach, pectoral and thoracic region translucent	Ventral	Haneda and Johnson (1962)
Family Pempheridae			
Parapriacanthus	Foregut, preanal duct, thoracic and abdominal organs, translucent thorax	Ventral	Haneda and Johnson (1962)
Suborder Trachinoidei			
Family Chiasmodontidae			
Pseudoscopelus	Mandibular, opercular and pectoral organs, body row organs	Ventral	Beebe (1932)
Order Lophiiformes			
Suborder Antennarioidei			
Family Chaunacidae			
Chaunax	Esca	Dorsal	Harvey (1952)
Family Ogcocephalidae			
Malthopsis, *Halicmetus* *Dibranchus*, *Dibranchichthys*, *Coelophrys*, *Halieutacea*, *Halieutopsis*, *Dibranchopsis*	Esca	Dorsal or midlateral	Harvey (1952); Garmann (1899)
Suborder Ceratioidei			
Families Melanocoetidae, Himantolophidae, Diceratiidae, Oneirodidae, Centrophrynidae, Ceratiidae, Gigantactinidae, Linophrynidae	Esca in females (sometimes one on 2nd cephalic ray)	Dorsal	Bertelsen (1951)
Ceratiidae (females only)	Caruncles on body	Dorsal	"
Ceratiidae (females only)	Caudal fin caruncles	Midlateral	"
Melanocetidae	Caudal rays	Midlateral	"
Linophrynidae (females only)	Hyoid barbel organs	Ventral	"
Linophrynidae	Larger teeth	Midlateral	"
Himantolophiidae	Tissue spots on head, body, and tail	Mainly ventral (9 out of 14 spots)	Beebe and Crane (1947)
Galatheathauma	Forked palatal organ	Dorsal	Wolff (1961)
Order Batrachoidiformes			
Family Batrachoididae			
Porichthys (incl. *Nautopaedium*)	Rows of photophores on body and head (larger and more numerous ventrally)	Mainly ventral	Hubbs and Schultz (1939); Greene (1899)

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[a] Bigelow and Schroeder (1948) misquote Burckhardt in referring to these as not functional.

[b] Mead and de Falla (1966) report a reflector near the pelvics in young *Rosenblattia* reminiscent of the luminous system found in other members of the family, but luminescence has not been observed.

Of the 42 families in Table I, in 29[2] the luminescence is entirely or predominantly ventral, in 12 entirely or predominantly dorsal, and in 1 mid-lateral. It is clear that in the majority of bioluminescent families, 69%, the luminous organs or tissues are predominantly ventral.

The order Lophiiformes contains most of the families with dorsal photophores. In these it is the esca which usually is luminous. The esca is mounted on a mobile illicium which is probably brought down to the mouth in the last phase of luring prey into its jaws. Hence, although it is anatomically dorsal, functionally it may be brought down to the mid-lateral or sometimes ventral position. Omitting the families whose sole luminous organ is in the esca, 86% have predominantly ventral luminous organs or tissues.

Only the following luminescent families have more than a dozen luminous species: Searsidae (15–20); all but one (Idiacanthidae) of the eight families of stomiatoids (about 200 in suborder); Myctophidae (well over 200 species in 30 genera); Macrouridae (about 1 or 2 doz); Ogcocephalidae (about 2 doz); and only one (Linophrynidae) of the eight families of ceratioids (suborder with about 80 species).

It is evident from the foregoing that, even though the majority of the 80 species of ceratioids have predominantly dorsal photophores, at least four or five times this number of luminescent species have predominantly ventral photophores.

The proportion of individuals with predominantly ventral photophores is probably even higher. In a study of the deepsea fish within an 8 mile circle south of Nonsuch Island, Bermuda, Beebe (1937) found that specimens of the genus *Cyclothone* (Gonostomatidae) comprised 82% of the 94,684 specimens of deepsea fish taken; these were followed in relative abundance of specimens by Myctophidae with 10%. Both these groups have predominantly ventral photophores and together comprise 92% of individuals taken. If these figures are generally applicable, and *Cyclothone* is generally considered to contain the largest number of individuals of any genus, then it is apparent that fishes with mainly ventral photophores predominate over those with mainly dorsal photophores in the realm of individuals, as well as in the realm of species and families. Based on evidence cited, one may generalize that photophores and luminous tissue predominate on the ventral half of fishes.

In addition to position of the photophores other factors are sometimes involved in directing the light from photophores downward. In certain cases the orientation and structure of the light organs may direct or focus rays downward or prevent outward or upward emmission of light. Greene (1899) states of *Porichthys*, "... where pigment is increased in amount on the dorsal side of the photophores. This would have the effect of blocking dorsal emission of the light from the lateral photophores. The lens is so placed in the lateral photophores to direct the light downwards at an angle, instead of horizontally."

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[2] This number may have to be reduced to 28 since although the luminous ducts in the Paralpididae are morphologically ventral the entire transparent fish glows and, further, the fishes have been observed to hold themselves in a vertical position (Rofen, 1966a).

Fraser-Brunner (1949) in his revision of the Myctophidae indicates, "It has often been stated that the light-organs of some forms, such as *Electrona* and *Diaphus*, are divided by a septum into upper and lower parts; but I find that the appearance of such division is given by the extension of the reflective layer with its dark edge downward on the inner side of the scale, forming a kind of screen over the upper half of the lens, no doubt deflecting the light downwards . . . . Lesser degrees of this condition are found in several other genera. A similar development is to be found in *Vinciguerria* and other Gonostomatids." Hickling (1928 *cited in* Harvey, 1952) reports that the luminescence visible from directly underneath *Spinax* (=*Centroscyllium*) disappears when it is rotated slightly laterally.

It would appear likely that a considerable selective pressure has been acting during the evolution of these luminous organs and tissues, influencing their position, orientation, and structure so that the light they emit will be directed downwards. Before dealing directly with the selective pressures involved, the specific functions of photophores and luminous tissues will be examined to determine their influence on position. Some of the following theories on function are included only for the sake of completeness.

PHOTOPHORE FUNCTION

As photophore function has been discussed in more or less detail in the surveys mentioned in the introduction, the discussion here of function is abbreviated. In some species at least, it is probable that the luminous tissues or organs are polyfunctional.

INTRASPECIFIC FUNCTIONS

The following involve interactions between members of the same species.

1. To lure or attract potential mates (Harvey, 1957, p. 364).
2. To enable selection of mate of the right species — photophores and luminous tissues are often different in different species and so could act as recognition signs (Harvey, 1952, p. 552; Marshall, 1954, p. 281).
3. To indicate sex of the possessor (Marshall, 1954, p. 284; Nicol, 1960, p. 578).
4. To aid in schooling (Marshall, 1954, p. 168, 281).[3]
5. For use in courtship displays (Bolin, 1961, p. 38; Crane, 1965, p. 239).
6. To space individuals evenly to utilize food or other resources, it not being possible to establish territories in mid-water where there are no fixed reference points (present study).[4]

INTERSPECIFIC FUNCTIONS

The following involve securing food.

1. To lure prey (Harvey, 1952, p. 553; Marshall, 1954, p. 267, 275) with esca, buccal, and body organs.

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[3] The lanternfish *Gonichthys coccoi* may form dense schools at night (Marshall, 1954).

[4] Only one genus of strictly benthic habits is bioluminescent — *Porichthys;* the rest reside in mid-water.

2. To illuminate prey (Marshall, 1954, p. 273) — orbital and cheek organs act as headlights.

3. Illuminating retina to lower the threshold to stimulate eye (Marshall, 1954, p. 288),[5] or to adapt the eye so it would not be blinded by brighter flashes.

The following involve avoiding predators.

1. To startle or confuse predators (Harvey, 1952, p. 552; Nicol, 1958, p. 727; Nicol, 1960, p. 577; Marshall, 1954, p. 286) with bright flashes or luminous clouds.

2. To divert predators from females (Bolin, 1961, p. 38), which are reproductively more valuable than males (this assumes that a male can fertilize more than one female or that males are more numerous to start with).

3. To reveal predators to other individuals (survival value to the species, not necessarily to the individual): (*a*) by illuminating the external surface of a nearby predator, thereby revealing it to larger predators or to members of the prey species (Nicol, 1960, p. 576; Burkenroad, 1943, p. 161). This is known as the 'burglar alarm' effect; (*b*) by luminescing when swallowed and making the predator glow, revealing the predator to the prey species or to larger predators (present study; see discussion below on counter-strategy).

4. To mislead predators through mimicry. Springer (1957) suggests that *Porichthys porissimus* mimics a ctenophore, and Robins and de Sylva (1965) suggest that the pelvic organ of *Kasidoron* mimics a siphonophore. The latter appears structurally and ecologically likely, but luminescence has not yet been observed.

PHOTOPHORE PLACEMENT

EFFECT OF SPECIFIC FUNCTIONS

Before passing on to the general theories on the distribution of luminescence on the body, the possible influence of specific photophore functions on position will be examined. None of the functions mentioned above under Intraspecific Functions would appear to require limitation of luminescence to the venter. One restriction in placement that these functions might impose is visibility in lateral view, as photophores used in social communication should be visible to members of its own species living at about the same depth. But lateral rather than ventral luminescence would appear to be imposed by this requirement.

In attracting prey there would not seem to be any overall restriction in dorso-ventral placement of luminous lures. The lures are dorsal in the lophiiforms (esca) and chauliodontids and ventral in the stomiatoids (mental barbel light organs). However, these are situated near the anterior end of the fish so the prey can be lured close to the jaws or, as in the "mouse-trap" arrangement of *Galtheathauma*, within the mouth.

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[5] This theory appears somewhat doubtful to the author. Light from the fishes own photophore would appear to contribute to visual "noise" and reduce information content, resulting in a less sharp image. Retinal divercula have now been found to function as visual receptors rather than as photophores (Munk, 1966).

The headlight organs, to function effectively in illuminating prey so they may be seen by the eyes, are of necessity placed near the eye. The eyes are usually dorsal or mid-lateral and this influences the dorso-ventral positioning of headlights.

Organs possibly used in startling, confusing, or diverting predators comprise the following: (*a*) dorsal stern chasers in certain male myctophids (Bolin, 1961); (*b*) the shoulder organ of searsids, which Parr (1960) suggests secretes or ejects a luminous substance and which Nicol (1958) observed discharging a bright luminous cloud when the specimen (*Searsia*) was handled; (*c*) the anal gland of *Malacocephalus* (Macrouridae), which Nicol (1960) indicates ejects a luminous cloud; (*d*) the dorsal and mid-lateral caruncles of ceratiids, which can luminesce only through the emission of a secretion (pressing the median dorsal caruncles results in the pouring out of a whitish yellow thread which breaks up and spreads into numerous points of light according to Bertelsen (1951)). These organs tend to be dorsal but the sample is too small to be significant. There appears to be no inherent reason for predator-revealing photophores to be either dorsal or ventral. Siphonophore mimicry (possible in *Kasidoron*), for realistic imitation, would need to be ventral.

As the specific functions attributed to bioluminescence do not seem to require predominantly ventral position one must look elsewhere for explanation of this pattern.

GENERAL THEORIES ON POSITION

Two theories have been advanced to explain ventral luminescence. These are discussed below, following which a third theory is developed.

Marshall (1954, p. 277) has advanced a theory to explain the predominantly ventral emission of light. He suggests that if photophores were dorsally placed the fish's position might be given away to predators by the reflection of sunlight from the silvery reflectors of the photophores. This theory would appear to have merit in regard to those fishes which inhabit the photic zone during the day and possess photophore reflectors.

Harvey (1952), however, reports that of the fish with open glands containing luminous bacteria a few species have reflectors and some lack them. The Gadiformes without reflectors still have ventral luminous organs. Harvey (1952, 1957) and Iwai (1960b) do not report reflectors in the Elasmobranchii but the luminous organs and tissues are still mainly ventral in four out of five genera. Reflectors may be involved in selection for ventral light organs in fish that have reflectors. But another factor must be involved in fishes which lack reflectors, yet which still have predominantly ventral light organs.

Clarke (1963) has recently developed another theory (apparently first proposed by Jerzmańska, 1960; *see also* Aleev, 1963 in Parin, 1964), that of "photophore countershading," to explain the ventral location and emission of photophores in squids, crustaceans, and fishes. He points out that this theory is compatible with other theories and leaves open the possibility that photophores may have different functions in different environmental situations. He suggests that an organism lacking ventral photophores would be sharply silhouetted against the downcoming sunlight while in the photic zone. By distributing photophores over the ventral surface of the body and matching the luminescence to the down-coming sunlight, the silhouette is destroyed. This phenomenon might be referred to as disruptive illumination as a parallel to disruptive colouration, both phenomena serving to make the animals less conspicuous in their environment (countershading does not appear too apt a term). Clarke's theory provides an explanation for the rather similar rows of photophores along the underside of many fishes, e.g. stomiatoids, myctophoids, and chiasmodontids. A row along the entire underside is necessary to disrupt the whole silhouette. It might well also apply to those acanthopterygians with internal light organs and transparent venters — Trachichthyidae, Leiognathidae, Apogonidae, and Acropomatidae. This theory would also explain why fishes living deeper than sunlight penetrates, have photophores poorly developed or lacking.

There remain, however, some examples which this theory does not satisfy. Species of *Porichthys* are benthic (Clemens and Wilby, 1961) rather than pelagic. Hence they do not require silhouette disruption. The luminescence of some taxa is not arranged in a manner to provide disruptive illumination. The organs of gadiforms, pempherids, and cetomimids are too localized to provide disruption of the entire silhouette. Clarke also considers that "a large number of predators hunt from below as indicated by their permanently upwardly directed eyes." However, the number of genera and individuals with tubular eyes at least angled upwards if not vertical is not large (see list in next section). If there were many predators with upwardly directed eyes it would follow that fishes with ventral luminescence would extinguish the light at night, or otherwise be exposed to the "large number of predators hunting from below." Yet Harvey (1952) mentions instances of *Isistius*, *Chauliodus*, and *Myctophum* emitting light in otherwise dark laboratories. Zahl (1953) reported that self-generated light was most often seen in specimens collected during night fishing at Messina. Clarke and Denton (1962) report that at times and depths (at night or at deeper levels during the day) at which only weak illumination is received from the sun, bioluminescence contributes a significant portion of the total light present (of course not all this can be attributed to fishes). These observations fit in with many of the specific functions of bioluminescence. To be of use in mate selection, luring prey, etc. the light organs must stand out against the background, i.e. shine more strongly than the ambient light. The silhouette may also be reduced by increasing compression and reflectivity of the body (Denton and Nicol, 1965).

The above discussion suggests that an additional explanation is required to account for at least certain cases of predominantly ventral luminescence, although the above theories may apply in the stated situations. Bertelsen (1958) has suggested *Opisthoproctus* may have a downwardly directed light organ so that predators with horizontally inclined eyes would not see its light. This idea is expanded here into a general principle, applying to most luminous fishes. It is, further, coupled with several bathymetric clines. A greater number of both individuals and species of predators live in the upper waters than in the depths. The clines may be demonstrated by the following figures. Whereas many hundreds of species are known from the upper waters of the ocean, only 300 have been reported below 2000 m, and less than 38 below 3660 m (Grey, 1956). In addition both the size and number of individuals tend to be greater in upper waters. Because of these clines there is a greater probability that a fish would be seen and consumed by predators if its bioluminescence was dorsal rather than ventral (Fig. 3).

It is worthwhile to consider the site of predation in more detail. Some fishes, such as *Xiphias* and certain scombrids, are eurybathic and dive to considerable depths, at times, to secure their prey. But most predators preying on luminous fishes probably restrict themselves to prey within a few hundred metres above or below them at any one time. The sighting and hot pursuit of individual prey up or down for great distances is restricted not only by the limits of perception,[6] but also by problems of energy and buoyancy. This limitation would reduce the up and down difference in potential predation. But even slight selective pressures applied over long periods of time will effect evolutionary change.

This potential difference in predation is increased, however, by diurnal migration of bathypelagic fishes. These fishes, like most of the bathypelagic fauna, undergo vertical migrations daily, ascending to shallower depths at night, then returning to deeper depths for the day. The nightly ascent towards the more densely populated upper waters considerably increases the predation potential from above. This factor reinforces selection for ventral bioluminescence.

Usually, the taxa contravening the general rule of ventral luminescence are fairly large as compared with most mesopelagic and deepsea fishes (over a foot in length (*Somniosus*, *Saccopharynx*, *Eurypharynx*, some lophiiforms)) and hence have less fear of predation, or their luminous organs are designed to attract fishes and may be turned off (lophiiforms). *Bathylaco*, however, is quite small, attaining only 215 mm. But the dorsal position of its photophore is probably due to a headlight function as it is immediately anterior to the eye.

In conclusion one may suggest that luminous organs and tissues are predominantly positioned and focussed ventrally because of three factors —

(1) Ventral photophores with reflectors do not disclose fishes to predators above them by reflection of sunlight during the day;

(2) Ventral photophores and luminous tissues may disrupt the silhouette of mesopelagic fish during the day and avoid disclosure to those predators which are below them;

(3) Ventrally directed luminescence is less likely to subject the fish to

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[6] The distance the predator can see its luminous prey has some bearing on the degree of selection: the greater distance the predator can see, the greater degree of predation the prey will suffer. Nicol (1958) calculates that the human eye could detect a luminescing *Myctophum punctatum* at distances of up to 10–16 m. Clarke and Denton (1962) suggest that the eyes of deepsea fishes may be expected to be between 10 and 100 times more sensitive than the human eye. Maximum sighting distance is not a simple product of these two factors, but a sighting distance up to at least 50 m does not seem unreasonable for clear oceanic waters (Nicol, 1963).

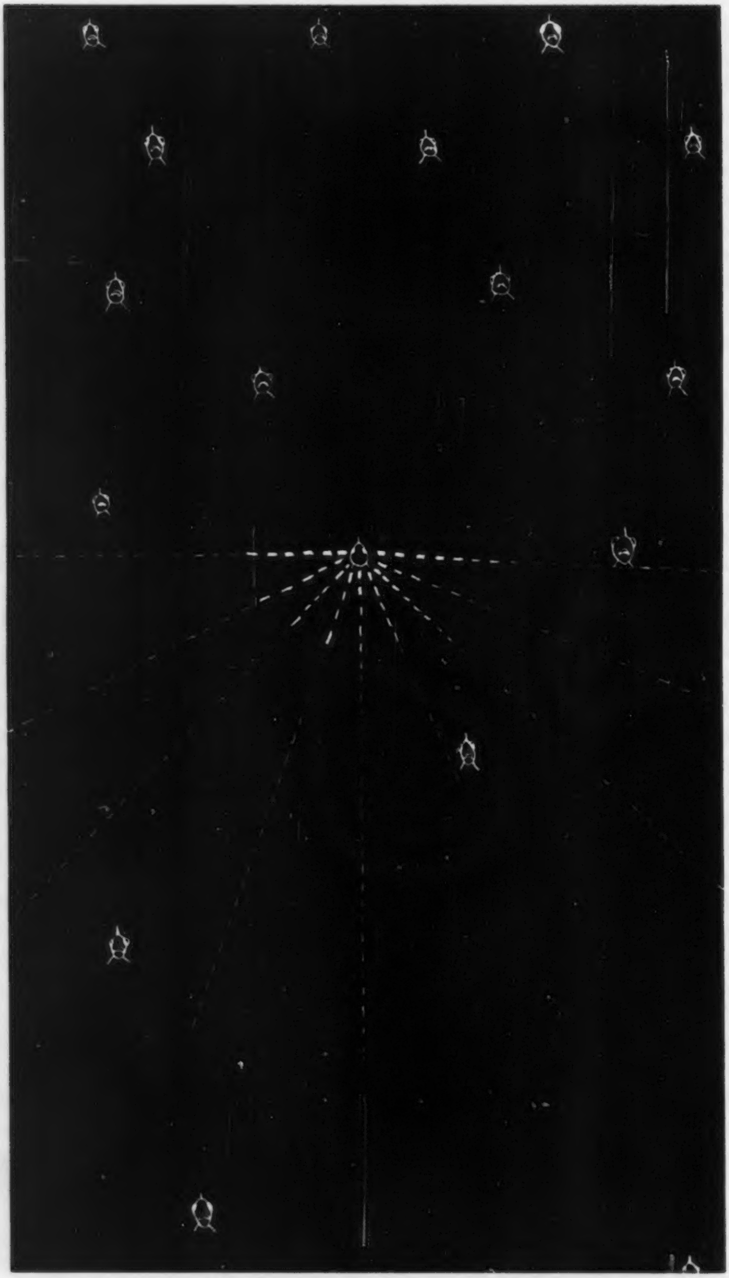

FIG. 3. Bioluminescence may expose an individual to predators. Ventral bioluminescence is selected for because of the lower number of predators below (effect exaggerated for purpose of illustration).

predation during twilight and night since there are fewer predators below than above in the mesopelagic habitat and since fishes at the bottom will not be seen by predators above them.

A program of observations on luminous fishes from a bathysphere or submarine throughout the diurnal migration would be very profitable in regard to bioluminescence. Observations could be made on social, feeding, and predatory behaviour and body orientation in relation to illumination of light organs and tissues at different hours, and whether reflectors are screened in daylight. This could be supplemented by shipboard operations such as echo-sounding and midwater trawling, with determination of light values at various levels. Barham (1966) describes a similar study of movement and composition of the deep scattering layer from a diving saucer, but observations on bioluminescence were not reported.

COUNTER-STRATEGY

Evolution can at times be likened to the game of chess wherein a new strategy may eventually be matched by a counter-strategy. The development of luminescence for specific functions has resulted in counter-strategies to avoid or take advantage of the consequences. Some bioluminescent counter-strategies will be briefly considered here.

Fishes can be illuminated by headlight organs of predators and by burglar-alarm luminescence. This has been countered in many fishes by the evolution of a light-absorbing pigment. In the twilight zone and deeper are found fishes characterized by black and red pigments (Marshall, 1954, p. 163). These pigments would absorb the predominantly blue light of attenuated sunlight and bioluminescence and so render them inconspicuous (Clarke and Denton, 1962). Nicol (1958) has demonstrated that the reflectivity of epidermis of *Chauliodus*, *Cyclothone*, and *Xenodermichthys* is very low (2–4%), although that of *Argyropelecus*, which is silvery, is high (40–65%).

Fishes would be at a disadvantage if exposed to predators by the light of luminescent organisms they had swallowed, or if this glow warned other prey. Many taxa of oceanic and deepsea fish possess black peritoneum, gastrointestinal canals, or stomachs. This pigmentation has been suggested to conceal ingested luminescent prey (McAllister, 1961; Walters, 1964). Dark external pigment would also assist in this regard. Another tactic is found in the trachichthyid *Parapriacanthus*. It *uses* the luminescence of its prey (Haneda and Johnson, 1962). Luminescent material from ingested *Cypridina* (an ostracod) is stored in the pyloric caeca and displayed in a thoracic luminous organ whose luminescence is controlled with chromaophores.

With the origin of many taxa having predominantly ventral luminescence there has evolved a small group of specialized predators which take advantage of this phenomenon (Marshall, 1954, p. 228; Walls, 1942, p. 402). These have tubular eyes admirably constructed to detect downcoming light from luminescent fishes (or silhouettes of non-luminescent fishes). The genera with upwardly directed tubular eyes are: *Argyropelecus*, *Ichthyococcus*, *Macropinna*, *Rhynchohyalus*, *Dolichopteryx*, *Opisthoproctus*, *Benthalbella*, *Phanops*, *Scopelarchoides*, *Neoscopelarchoides* (dorsal or anterodorsal tubular eyes), *Scopelarchus*, *Coccorella*, and *Evermannella* (Cohen, 1964a; Grey, 1964; Marshall, 1954, 1955; Parr, 1928; Rofen, 1966b, c; not all species in all these genera have tubular eyes).

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REFERENCES

ALCOCK, A. 1902. A naturalist in Indian seas. John Murray, London. 328 p.

BARHAM, ERIC G. 1966. Deep scattering layer migration and composition: observations from a diving saucer. Science, **151**: 1399–1403.

BEEBE, WILLIAM. 1932. Nineteen new species and four post-larval deep-sea fish. Zoologica, **13**(4): 47–107.
1933a. Deep-sea fishes of the Bermuda oceanographic expeditions. Family Alepocephalidae. Ibid., **16**(2): 15–93.
1933b. Deep-sea fishes of the Bermuda oceanographic expeditions. Introduction. Ibid., **16**(1): 5–11.
1933c. Deep-sea fishes of the Bermuda oceanographic expeditions. Family Argentinidae. Ibid., **16**(3): 97–147.
1937. Preliminary list of Bermuda deep-sea fish. Ibid., **22**(3): 197–208.

BEEBE, WILLIAM, AND J. CRANE. 1939. Deep-sea fishes of the Bermuda oceanographic expeditions. Family Melanostomiatidae. Ibid., **24**(6): 65–238.
1947. Eastern Pacific expeditions of the New York Zoological Society. XXXVII. Deep-sea ceratioid fishes. Ibid., **31**(10): 151–182.

BERTELSEN, E. 1951. The ceratioid fishes. Dana-Rept. No. 39: 1–276.
1958. A new type of light organ in the deep-sea fish, *Opisthoproctus*. Nature, **181**: 862–863.

BERTELSEN, E., AND O. MUNK. 1964. Rectal light organs in the argentinoid fishes *Opisthoproctus* and *Winteria*. Dana-Rept. No. 62: 1–17.

BERTIN, LEON. 1934. Les poissons apodes appartenant au sous-ordre des Lyomères. Ibid., No. 3: 1–56.

BIGELOW, HENRY B. 1964. Bathylaconoidea. Fishes of the western North Arlantic. Mem. Found. Marine Res. No. 1 (Pt. 4): 561–565.

BIGELOW, HENRY B., AND W. C. SCHROEDER. 1948. Sharks. Fishes of the western North Atlantic. Mem. Sears. Found. Marine Res. No. 1 (Pt. 1): 1–576.

BOLIN, ROLF L. 1961. The function of the luminous organs of deep-sea fishes. Proc. Ninth Pacific Sci. Congr. 1957, **10**: 37–39.
1966. Interim account of the family Myctophidae. Fishes of the western North Atlantic. Mem. Found. Marine Res. No. 1 (Pt. 5): 190–191.

BURCKHARDT, RUD. 1900. On the luminous organs of selachian fishes. Ann. Mag. Nat. Hist. Ser. 7, **6**: 558–568.

BURKENROAD, M. D. 1943. A possible function of bioluminescence. J. Marine Res., **5**: 161–164.

CLARKE, WILLIAM D. 1963. Function of bioluminescence in mesopelagic organisms. Nature, **198**(4887): 1244–1246.

CLARKE, G. L., AND E. J. DENTON. 1962. Light and animal life. *In* M. N. Hill [ed.] The sea. Vol. 1. Interscience Publishers, New York, London. p. 446–468.

CLEMENS, W. A., AND G. V. WILBY. 1961. Fishes of the Pacific coast of Canada. Bull. Fish. Res. Bd. Canada, No. 68. 443 p.

COHEN, DANIEL M. 1964a. Bioluminescence in the Gulf of Mexico anacanthine fish *Steindachneria argentea*. Copeia, No. 2: 406–409.
1964b. Suborder Argentinoidea. Fishes of the western North Atlantic. Mem. Sears Found. Marine Res. No. 1 (Pt. 4): 1–70.

CRANE, JR., JULES M. 1965. Bioluminescent courtship display in the teleost *Porichthys notatus*. Copeia, No. 2: 239–241.

DENTON, E. J., AND J. A. C. NICOL. 1965. Reflexion of light by external surfaces of the herring, *Clupea harengus*. J. Marine Biol. Assoc. U.K., **45**: 711–738.

FRASER-BRUNNER, A. 1949. A classification of the fishes of the family Myctophidae. Proc. Zool. Soc. London, **118**(Pt. 4): 1019–1106.

GARMANN, S. 1899. The fishes. Mem. Museum Comp. Zool., **24**: 1–431.

GREENE, CHARLES WILSON. 1899. The phosphorescent organs in the toadfish, *Porichthys notatus* Girard. J. Morphol., **15**(3): 667–692.

GREY, MARION. 1956. The distribution of fishes found below a depth of 2000 metres. Fieldiana Zool., **36**(2): 75–337.
1964. Family Gonostomatidae. Fishes of the western North Atlantic. Mem. Sears. Found. Marine Res. No. 1 (Pt. 5): 78–240.

HANEDA, Y. 1950. *Harpodon nehereus*, a non-luminous fish. Pacific Sci., **4**: 135–138.
1951. The luminescence of the deep-sea fishes, families Gadidae and Macrouridae. Ibid., **5**: 372–378.
1958. Preliminary report on a luminous fish of the family Paralepididae. Sci. Rept. Yokosuka City Museum, **3**: 31–34.

HANADA, Y., AND FRANK H. JOHNSON. 1962. The photogenic organs of *Parapriacanthus beryciformes* Franz and other fish with the indirect type of luminescent system. J. Morphol., **110**(2): 187–198.

HARRY, R. R. 1952. Deep-sea fishes of the Bermuda oceanographic expeditions. Families Centomimidae and Rondeletiidae. Zoologica, **3** (Pt. 1): 55–72.

HARVEY, EDMUND NEWTON. 1952. Bioluminescence. Academic Press Inc., New York. 649 p.
1957. The luminous organs of fishes. *In* M. E. Brown [ed.] The physiology of fishes. Vol. 2. Academic Press, Inc., New York. p. 345–366.

HUBBS, CARL L., AND LEONARD P. SCHULTZ. 1939. A revision of the toadfishes referred to *Porichthys* and related genera. Proc. U.S. Natl. Museum, **86**(3060): 473–496.

IWAI, TAMOTSU. 1958. A study of the luminous organ of the apogonid fish *Siphamia versicolor* (Smith and Radcliff). J. Wash. Acad. Sci., **48**(8): 267–270.
1960a. Notes on the luminous organ of the apogonid fish, *Siphamia majimai*. Ann. Mag. Nat. Hist. Ser. 13, **2**: 545–550.
1960b. Luminous organs of the deep-sea squaloid shark, *Centroscyllium ritteri* Jordan and Fowler. Pacific Sci., **14**(1): 51–54.

JERZMAŃSKA, ANNA. 1960. (The structure and biological significance of light organs in the Teleostei). Przeglad Zoologiczny, **4**: 112–118. (In Polish.)

MARSHALL, N. B. 1954. Aspects of deep-sea biology. Philosophical Library, New York. 380 p.
1955. Alepisauroid fishes. Discovery Rept., **27**: 303–336.

McAllister, D. E. 1961. A collection of oceanic fishes from off British Columbia with a discussion of the evolution of black peritoneum. Bull. Natl. Museum Canada, No. 172. p. 39–43.

1967. The evolution of branchiostegals, and associated gular, opercular and hyoid bones in teleostome fishes. Ibid. (In press.)

Mead, Giles W., and J. E. de Falla. 1966. New oceanic cheilodipterid fishes from the Indian Ocean. Bull. Museum Comp. Zool., **134**(7): 261–274.

Misra, K. S. 1953. An aid to the identification of the fishes of India, Burma, and Ceylon. II. Clupeiformes, Bathyclupeiformes, Galaxiiformes, Scopeliformes and Ateleopiformes. Records Indian Museum, **50**(3/4): 367–422.

Munk, O. 1959. The eyes of *Ipnops murrayi* Günther, 1878. Galathea Rept., **3**: 79–87.

1966. Ocular anatomy of some deep-sea teleosts. Dana.-Rept. No. 70: 1–62.

Nicol, J. A. Colin. 1958. Observations on luminescence in pelagic animals. J. Marine Biol. Assoc. U.K., **37**: 705–752.

1960. The biology of marine animals Sir Isaac Pitman & Sons Ltd., London. 707 p.

1963. Some aspects of photoreception and vision in fishes. *In* F. S. Russell [ed.] Advances in marine biology. Vol. 1. Academic Press, London and New York. p. 171–208.

Parin, N. V. 1964. Data on the biology and distribution of the pelagic sharks *Euprotomicrus bispinatus* and *Isistius brasiliensis.* Akad. Nauk SSSR, Trud. Inst. Oceanol., **73**: 173–195. (English Transl., Israel Program Sci. Transl., 1966.)

Parr, Albert Eide. 1928. Deepsea fishes of the order Iniomi from the waters around the Bahama and Bermuda Islands. Bull. Bingham Oceanogr. Coll. **3**(art. 3): 1–193.

1951. Preliminary revision of the Alepocephalidae, with the introduction of a new family, Searsidae. Am. Museum Novitates, No. 1531: 1–21.

1960. The fishes of the family Searsidae. Dana.-Rept. No. 51: 1–109.

Robins, Richard C., and Donald P. de Sylva. 1965. The Kasidoroidae, a new family of mirapinniform fishes from the western Atlantic Ocean. Bull. Marine Sci. Gulf Caribbean, **15**(1): 189–201.

Rofen, Robert R. 1966a. Family Paralepididae. Fishes of the western North Atlantic. Mem. Sears. Found. Marine Res. No. 1 (Pt. 5): 205–461.

1966b. Family Evermannellidae. Fishes of the western North Atlantic. Ibid. No. 1 (Pt. 5): 511–565.

1966c. Family Scopelarchidae. Fishes of the western North Atlantic. Ibid. No. 1 (Pt. 5): 566–602.

Springer, Victor G. 1957. Mysterious midshipman . . . little known curiosity of the sea. Texas Game Fish., Nov. 1957: 6–7.

Walls, Gordon Lynn. 1942. The vertebrate eye and its adaptive radiation. Cranbrook Inst. Sci. Bull. No. 19: 1–785.

Walters, Vladimir. 1964. Order Giganturoidei. Fishes of the western North Atlantic. Mem. Sears. Found. Marine Res. No. 1 (Pt. 4): 566–577.

Wolff, Torben. 1961. Animal life from a single abyssal trawling. Galathea Rept., **5**: 129-162.

Zahl, Paul A. 1954. Fishing in the whirlpool of Charybdis. Natl. Geogr. Mag., **104**(5): 579–618.

The Zooplankton of Tanquary Fjord, Ellesmere Island, with Special Reference to Calanoid Copepods[1]

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ABSTRACT

Tanquary Fjord is ice-covered for a large part of the summer and hence very unproductive. The zooplankton showed variation between the years 1963 and 1964 both in abundance and in species composition, suggesting an unstable system. Development rates and breeding cycles of the calanoid copepods were indicated by the numbers in the various developmental stages. Two forms of *Pseudocalanus*, differing in the relation of urosome to cephalothorax length, were found. It is unknown whether the two forms are reproductively isolated. The dominant form has a biennial life cycle. *Microcalanus pygmaeus* appeared to breed biennially. The species *Calanus glacialis* and *C. hyperboreus* were shown to be opportunistic breeders; they developed to maturity over a period of several years.

INTRODUCTION

Investigations of copepod breeding cycles from various locations in the arctic have shown that the development rate is commonly lower than that exhibited by the same species in temperate waters (Digby, 1954; Fontaine, 1955; Grainger, 1959). From the high Arctic, however, there are few studies on this subject and the published data are meager (Bogorov, 1946; Brodsky and Nikitin, 1955; Johnson, 1963; Grainger, 1965). As a result, it is still unknown to what extent the polar environment affects the development rates and the breeding cycles of most copepods.

This investigation of copepod development was based on plankton collections taken at Tanquary Fjord (81°N) during the summers of 1963 and 1964. The genus *Pseudocalanus*, because of its abundance and convenient size, was chosen to be investigated in particular detail. Since there was an indication that two forms of *Pseudocalanus* occurred together, the morphological variation within this species was examined.

Tanquary Fjord is the most inland extension of the Greely Fjord system of northern Ellesmere Island. A map of the area is given by Ford and Hattersley-Smith (1965).

MATERIALS AND METHODS

The field work was carried out for the main part near the center of the fjord through a hole in the ice, approximately 6 miles from the head of the fjord.

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After mid-July, due to a deterioration of the ice, samples were taken either from loose ice with the aid of a small aluminum canoe or from a 22-ft cargo canoe equipped with an outboard motor.

A Nansen reversing bottle was used to take temperature readings and water samples for salinity and oxygen analysis. Oxygen analysis was by the Winkler method.

During 1963 plankton samples were taken by net tows from a variety of depths and locations. These samples are therefore not truly comparable with those of 1964, which were taken from standard depths. The 1963 samples were taken with nylon plankton nets of No. 6 (.239 mm) and No. 0 (.569 mm) mesh. Where frequencies of copepod developmental stages from both 1963 and 1964 are represented together on the same graph, the sample from 1963 was used which most closely approximated the depth of the corresponding sample from 1964.

During the summer of 1964, collections were taken at weekly intervals using nets of No. 20 (.076 mm), No. 6, and No. 0 mesh. All net tows were vertical and hauled by a hand winch at approximately 50 m per min. Later in the season when the ice had deteriorated, an attempt was made to return as close to the original station as ice conditions permited.

Each sample consisted of a single vertical haul and in all cases the abundance of developmental stages of the copepods is reported as numbers per tow. The developmental stages of *Pseudocalanus*, *Calanus glacialis*, *C. hyperboreus*, and *Microcalanus pygmaeus* were counted, as follows, from samples of each sampling date and the changes in numbers of the various stages (C = copepodite, N = nauplii) were used as an indication of development rate:

Adults and copepodite stages C3, C4, and C5 of *Pseudocalanus* were counted from the 1964 No. 6 net tows from depths of 10, 20, 50, and 100 m. The concentration of individuals present between 10 and 20 m was calculated by subtracting the number of animals in a 10-m sample from the number in the corresponding 20-m sample. Similarly the concentration between 20 and 50 was found by subtracting the number in a 20-m sample from that of a 50-m sample. Concentrations between 50 and 100 m are not included in Fig. 3, the numbers being too small to be meaningfully represented. In making these subtractions, several results were negative, that is, there were more individuals in the shallow tows than there were in the corresponding deeper ones. The appropriate spaces on the graph are marked with negative values accordingly.

Pseudocalanus copepodite stages younger than C3 were not retained efficiently in the No. 6 net, therefore, all copepodite stages were counted from the No. 20 net samples from 50 m. These data are used in Fig. 4 to report the relative abundance of stages. All counts of naupliar stages of *Pseudocalanus* were made from subsamples taken from the No. 20 net samples from 50 m. After removal of all large material, each sample was diluted to 20 ml and well-mixed subsamples of 2 ml each were taken from them using an automatic pipette. Depending on the concentration of nauplii the subsampling procedure was repeated until from 20% to 40% of each sample had been examined. The total number of each stage was then calculated for each sample.

Only a few adult females were found bearing eggs. Because of this, the free eggs in each sample were counted and their numbers included with the stage frequencies.

Measurements of cephalothorax lengths of *Pseudocalanus* adults and copepodite stages C4 and C5 were taken to give a further indication of development in this species. These measurements were made with an ocular micrometer at a magnification of 40×. The adult females from June 15 and 22, 1964 clearly showed a bimodal distribution of size. The smaller individuals appeared to be more rounded at the anterior end of the cephalothorax, suggesting that possibly two forms were present. These animals were measured at a higher magnification (80×) and at the same time the urosome lengths were measured. These two dimensions were plotted as a scatter diagram in an attempt to give a more precise description of morphological variation within the two size-groups.

All stages of *M. pygmaeus* (naupliar and copepodite) were counted from subsamples as described for *Pseudocalanus* nauplii.

Developmental stages of *C. glacialis* and *C. hyperboreus* were counted from the No. 0 net samples from 100 m.

HYDROGRAPHY

The general hydrography of Tanquary Fjord has been described by Ford and Hattersley-Smith (1965). Representative profiles of temperature and salinity (Fig. 1) show that during the summer there occurs a progressive warming of the upper 25 to 30 m, the increase at 5 m being approximately 1.5 C.

ICE

The fjord ice in May 1964 was 2.5 m thick at the main sampling station. During the melt period, it became difficult to make accurate measurements. After breakup in July, however, large pans were still approximately 1.2 m thick. During both years studied, a large amount of this ice remained to last another season. In 1964 the snow cover upon the ice was lost rather abruptly due to a marked temperature increase during the first week in June. For a period of several days after this, an estimated 80% to 90% of the fjord ice was water-covered. The amount of radiation reaching the fjord waters was therefore greatly increased at this time (Hanson, 1961). After a few days, this layer ran off to a great extent, and the ice itself began to melt. The ice albedo would again rise considerably at this time. After this, there occurred a very gradual deterioration of the ice until breakup finally occurred in late July.

OXYGEN

The concentration of dissolved oxygen is given (Fig. 2) as a possible indication of phytoplankton production. It is probable that only the top few meters have any relevance to this study.

A high concentration of oxygen occurred in the top 10 m at the end of May, with a maximum of 14 ml/liter (supersaturation) just under the ice on

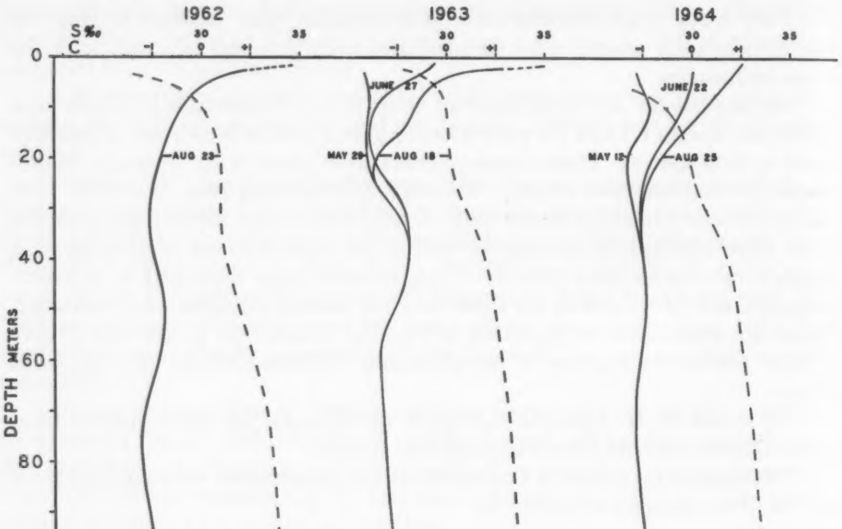

FIG. 1. Selected temperature (solid lines) and salinity (broken lines) profiles for Tanquary Fjord, 1962–64. Data from 1962 was published by the Canadian Oceanographic Data Center (1966).

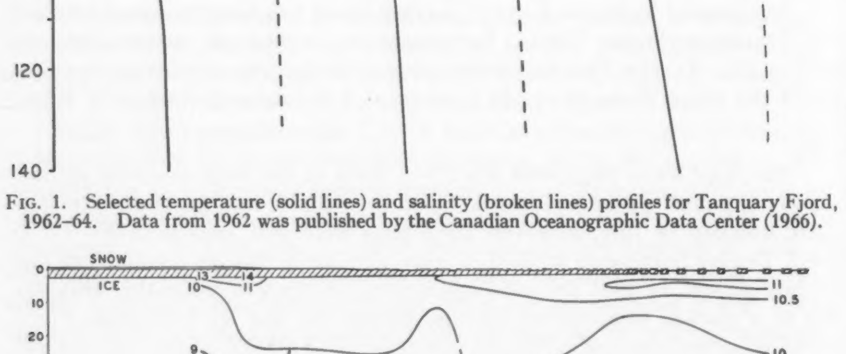

FIG. 2. Isopleths of dissolved oxygen (milliliter per liter). Each sampling date is indicated by a vertical mark on the abscissa. The depth of each isopleth was read from a concentration versus depth profile drawn for each sampling date.

May 31. This decreased rather abruptly as soon as the snow upon the ice melted the following week. These high concentrations of oxygen must be accounted for by abundant plant growth before the melt began.

It has been known for some time that the under surface of sea-ice is often covered with algal growth. Apollonio (1961) suggests that under-ice algae in the Arctic are adapted to low light intensities and that the production of these algae is inhibited by an increase in intensity. Such a phenomenon would help to explain both the high concentration of oxygen present before the snow-melt and the rapid disappearance of this layer when the transparency of the ice cover increased in early June.

Just before oxygen concentrations decreased in the top few meters, they increased in the 10 m to 20 m layer. This was presumably due to the advent of the spring growth of phytoplankton. During July and August, as the ice deteriorated, and broke up, there occurred an additional increase in oxygen concentration at the 5 m level.

Since the rapid increase in oxygen at the end of June was observed in the samples from only 1 day, these values are considered suspect.

ZOOPLANKTON

COPEPODA

The most abundant species of copepods at Tanquary Fjord were: *Oithona similis* (Claus), *Pseudocalanus* sp., *Microcalanus pygmaeus* G. O. Sars, *Oncaea borealis* G. O. Sars, *Calanus glacialis* Jashnov, and *Calanus hyperboreus* Krøyer. Of these, the two *Calanus* species accounted for the largest proportion by volume of most samples. All other species were responsible for a minor proportion of the total plankton per haul with the exception of *Metridia longa* (Lubbock). This species occurred in numbers comparable to those for *Calanus* in the 1963 collections, but was scarce in the 1964 material. Only a few specimens of *Scolecithricella minor* Brady occurred in 1963 but the species was found regularly in 1964. *Scolecithricella minor* adult males and adult females bearing spermatophores were found from late May until early August. Several copepodites but no adults of the two copepods *Pareuchaeta glacialis* (Hansen) and *Xanthocalanus* sp. were present in the 1963 collections but neither species was found in those from 1964.

MEDUSAE

The medusa *Aglantha digitale* O. F. Müller occurred in small numbers throughout the two seasons. The individuals collected on August 16, 1963 had a modal length of 5 mm and a maximum size of 7.5 mm. Only in early May did any specimens show gonads. The gonads never measured more than 1 mm in length. The specimens were somewhat smaller than those found by Grainger (1959) in Foxe Basin.

The only other medusa taken was *Aeginopsis laurenti* Brandt. Only three specimens, measuring from 5 mm to 7 mm in diameter, were observed.

CHAETOGNATHA

The chaetognath *Sagitta elegans* Verrill was found in considerable numbers in the 1963 collections and spawned during the latter part of that season. Length measurements of individuals from August 16, 1963 showed a bimodal frequency. The sample examined contained 433 immature individuals measuring from 7 mm to 30 mm with a mode of 15 mm, and an estimated 6000 newly spawned young measuring from 1 mm to 7 mm. No mature adults were observed; possibly the nets were not hauled at a sufficient speed to collect them. During 1964 *S. elegans* appeared in very reduced numbers with practically no newly spawned young.

The only other chaetognath found was *Eukrohnia hamata* (Mobius) of which a few specimens were taken in 1963.

AMPHIPODA

The amphipod *Parathemisto abyssorum* Boeck was the most abundant planktonic amphipod found. It was taken regularly in 1963 but only rarely in 1964.

PSEUDOCALANUS

Although previous studies have referred to the species common in the Canadian Arctic as *P. minutus* (Krøyer), there is some evidence suggesting that more than a single form is present. Closely related species have been described (see discussion in Wiborg, 1954); however, McLaren (1963) suggests that they have been described without proper consideration of ecophenotypic variation. He refers to the generic name alone and this practice is followed here.

DEPTH DISTRIBUTION

The depth distributions of *Pseudocalanus* copepodite stages C3, C4, C5 and adults (C6) are given (Fig. 3). The negative values indicated on the graph could have been the result of either a patchy distribution of plankton in the surface water or an active swimming of zooplankton away from the tow wire, or both.

The vertical movements indicated by Fig. 3, upward in spring and downward in autumn, agree essentially with those found in boreal waters (Ussing, 1938; Ostvedt, 1955).

The significance of the difference in abundance of copepodite stages between the tows of 50 m and 100 m was tested using a *t*-test for paired variates. Of the four stages only C5 was more abundant in the 100-m than in the 50-m tows ($p < .05-.02$) indicating a deeper distribution of this stage.

STAGE FREQUENCY

The frequency distributions of the developmental stages of *Pseudocalanus* were plotted against time (Fig. 4). It is evident that at all times there were two or three stage modes. In 1963 a group slowly progressed from C3 to C5 during the season. The large group of adults presumably spawned and died off by autumn, and the young produced by them developed to C2 by August. Follow-

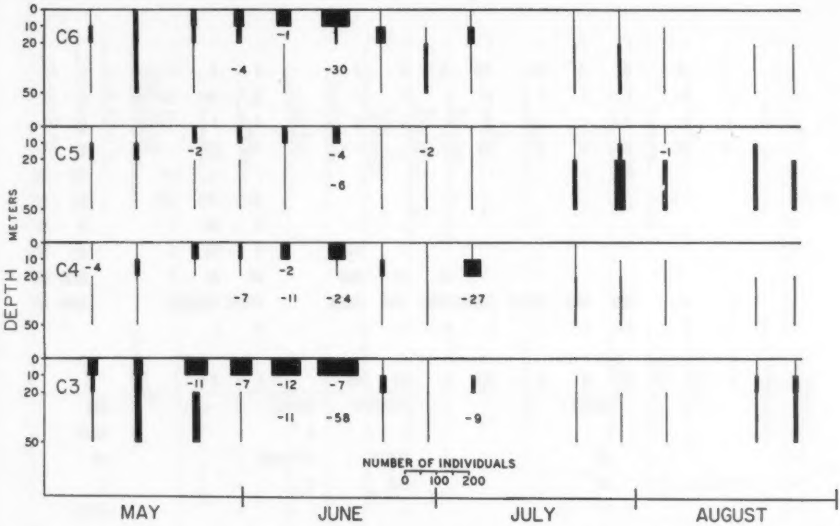

FIG. 3. Depth distributions of *Pseudocalanus* copepodites C3 to C6 from May to August 1964. Concentrations were calculated, by subtraction, from single vertical net hauls with an open net from 10, 20, 50, and 100 m. The density of animals below 50 m was too low to be meaningfully represented in the diagram. The areas of the cells are proportional to the concentrations of individuals.

ing the two surviving groups into the next year, we find that the C5 group developed into adults and the C2 group into C3. This development probably occurred in autumn while there was still sufficient food available to support growth. During 1964, the C3 group developed to C5 by the end of August and the adults spawned and died off, following the same pattern as in the previous year. The persistent mode at N3 developed to C1 in July and at the same time an apparently new mode appeared at N3. It appears also that egg laying continued throughout the summer; although the egg counts were variable the distinct modes of abundance among young stages suggest that there were two periods of increased egg production.

SIZE VARIATION

The length-frequency histograms (Fig. 5) show a bimodal size distribution for the C5 individuals during May and June of 1964. A bimodality of size is also suggested among the C4 individuals by their wide size range during May and June of 1964. Presumably the precision of measurement of these smaller animals was not great enough to indicate clearly the size modes. During June the smaller size group disappeared from the histograms of the immature stages and reappeared as a small size group among the adult females. This suggested that the smaller individuals were developing more rapidly than the larger at this time.

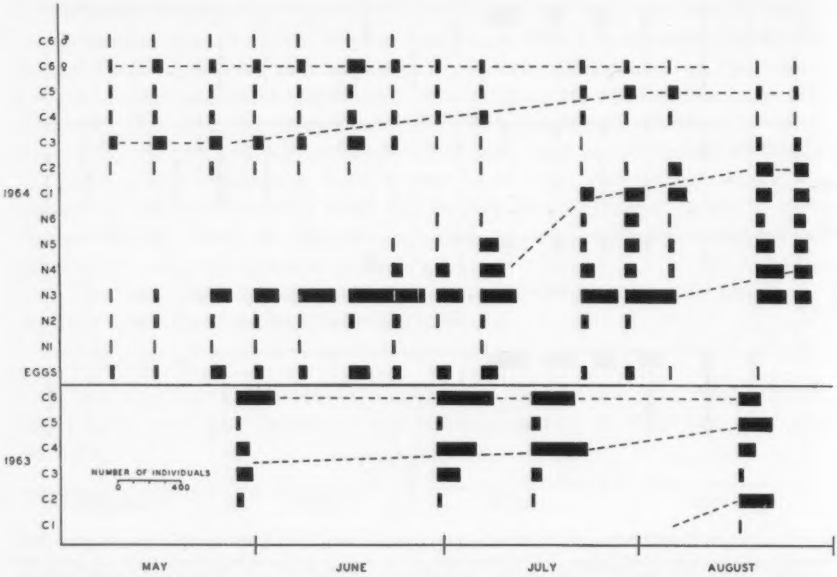

FIG. 4. Numbers of individuals in the various developmental stages of *Pseudocalanus* in single vertical net hauls from 50 m. Since the 1963 material was collected with a No. 6 mesh net, only copepodite stages are represented.

The scatter diagram relating urosome and cephalothorax lengths (Fig. 6) indicates that the larger and smaller forms show different linear relationships between these two characters. They can therefore be separated on the basis of this morphological variation.

MICROCALANUS

Very small numbers of *M. pygmaeus* nauplii were seen, although a few were counted in most samples including those of early May. The maximum number occurred in the sample of August 25.

The spawning period was evidently rather prolonged, as with *Pseudocalanus*. Only three adult males were observed, two in samples from May and one from July 6. Two mature females carrying spermatophores were seen, one on May 31 and the other on July 29.

The frequency distributions of the copepodite stages (Fig. 7) were in general similar to those of *Pseudocalanus*. The new generation appeared in July and the stage mode progressed to C3 by late August. An increase of adults on July 29 seems due to the development of a considerable proportion of the intermediate group to C6 while some remained as a mode of C4. The progression of the C4 mode became obscure in August and it seems possible that a large portion reached adulthood at that time. These individuals would thus have reached maturity in a period somewhat longer than 1 year.

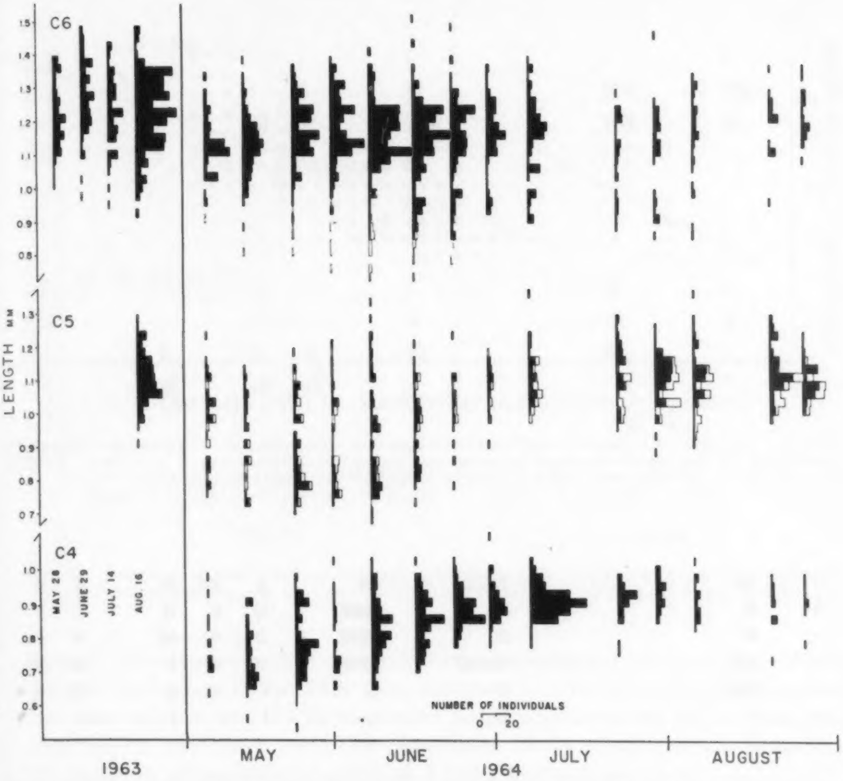

FIG. 5. Cephalothoracic lengths of *Pseudocalanus* copepodites C4 to C6 from May to August 1963 and 1964. Open cells indicate males, closed cells indicate females. Specimens were taken from vertical hauls of No. 6 mesh net.

CALANUS

The numbers of copepodites of both *C. glacialis* and *C. hyperboreus* found from May to August of 1963 and 1964 are given (Fig. 8). Assuming there is a minimum of growth during winter months, the stage mode of *C. glacialis* proceeded from C3 to C5 and C6 in a period of two summers. Most of the adult *C. glacialis* observed on June 15, 1964 showed developing ovaries, and some nauplii of this species were observed during most of the season. In *C. hyperboreus* a group at C4 developed to C5 in the 2 years, indicating a very slow rate of development.

DISCUSSION

It is apparent from the slow development rates exhibited by the copepods under study that Tanquary Fjord is a region of restricted production. Presum-

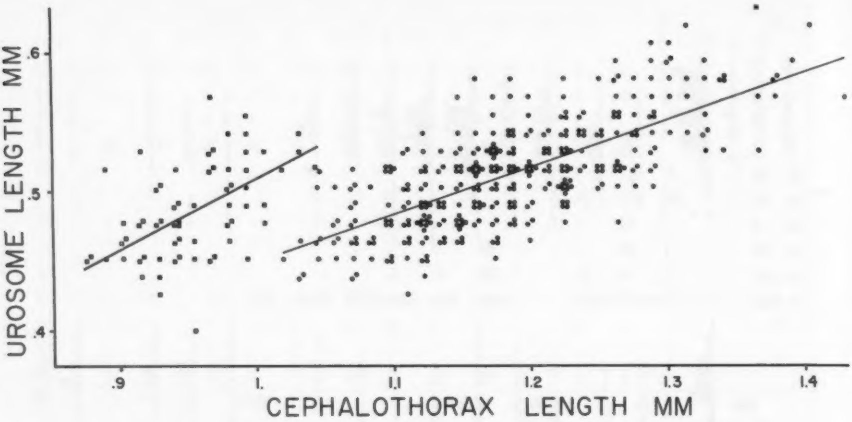

FIG. 6. Relations of urosome to cephalothorax length in *Pseudocalanus*. Regressions slopes and correlation coefficients for the small (squares) and large (circles) forms are: $b = .520, r = .906$ ($P = < .001$) and $b = .376, r = .984$ ($P = < .001$) respectively.

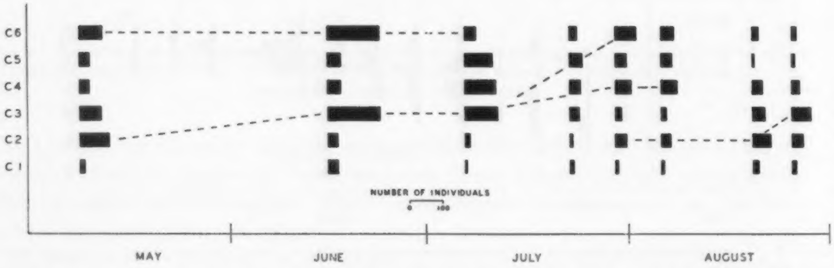

FIG. 7. Numbers of individuals of *Microcalanuss pygmaeus* in various copepodite stages from May to August 1964. Data from single vertical net hauls from 50 m.

ably the variation in species composition and in the numbers of individuals between years is an instability resulting from variable environmental conditions. During 1962 Tanquary Fjord was exceptionally ice-free (G. Hattersley-Smith, personal communication) possibly resulting in a marked increase in production. The samples obtained in 1963 were noticeably larger than those of the following year. Samples collected during 1965, although not considered here, showed an even greater reduction in biomass.

The breeding cycle of the copepod *Pseudocalanus* has been described from various areas (Ruud, 1929; Fish, 1936; Wiborg, 1944, 1954; Marshall, 1949; Digby, 1950, 1954; Fontaine, 1955; Grainger, 1959; McLaren, 1963, 1965; Carter, 1965). The breeding period or generation time has been estimated as being from 2½ months to 1½ years depending on the environment. In arctic

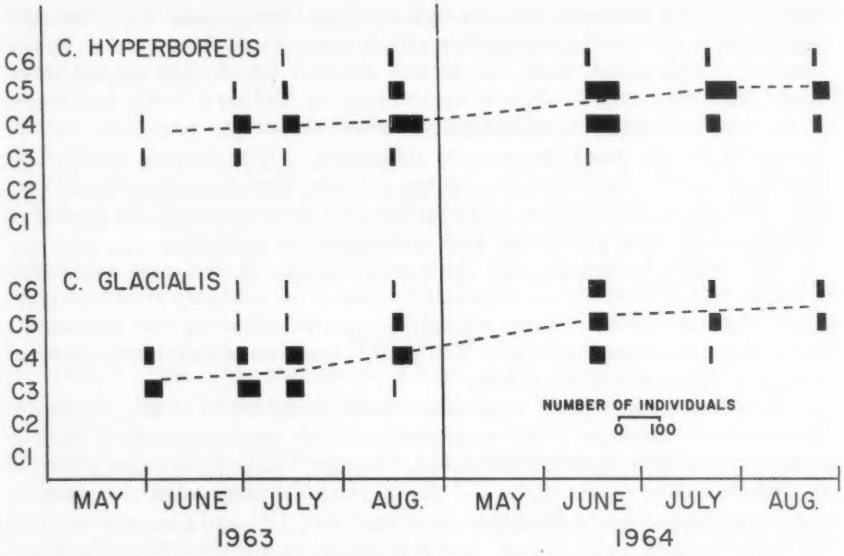

FIG. 8. Numbers of individuals of *Calanus glacialis* and *C. hyperboreus* in various copepodite stages from May to August 1963 and 1964. Data from single vertical net hauls from 100 m.

waters, *Pseudocalanus* has generally been considered basically an annual breeder. Tanquary Fjord is the most northern location at which *Pseudocalanus* has been studied and its development there appears to be slower than that reported for any other area. The progression of the stage modes indicate that the majority of individuals required 2 years to reach maturity.

Nearly constant low nauplii production and rapid development to N3 may have accounted for the persistent mode at N3 throughout the summer. Although egg production occurred throughout the season, two main broods seem to have been produced. This is characteristic of *Pseudocalanus* elsewhere (McLaren, 1963, 1965; Carter, 1965). The second brood, which only reached late naupliar stages by the end of the 1964 season, presumably died off during winter, as its counterpart was not represented at the beginning of the 1964 season. Nauplii of marine copepods do not normally overwinter perhaps because they have generally little resistance to food shortages and enviromental extremes (Marshall and Orr, 1955).

The disappearance of the smaller size group from the length-frequency histograms of both C4 and C5 in late June and the appearance of a group of small adults (Fig. 5) gives evidence of a small number of individuals which developed to adults at that time. Possibly this group completed its development within a period of 1 year and since the two groups appear to show a discontinuous separation in morphology, the two forms may represent reproductively isolated populations. However, the development of these smaller individuals was followed only through the last three stages. An alternative may be that the small individuals resulted from the persistance of a limited number of individuals from the second seasonal brood. The second brood would have developed in slightly warmer and, as McLaren (1965) has shown, at low environmental temperatures, marked phenotypic responses may be accounted for by small temperature differences. These animals would have been spawned in 1962 when, as pointed out, the fjord was exceptionally ice-free. As a result, an increase in production may have sustained the growth of animals which otherwise would have succumbed to starvation.

The length histograms also show polymodality in the main population of larger, presumably 2-year-old adult females. This probably reflects a polymodal size distribution of these animals in mature, indicating that the population is not of homogeneous origin. Rather, it is made up of individuals spawned at various times during the season.

The development of *M. pygmaeus* cannot be traced as clearly as that of *Pseudocalanus*. Grainger (1965) suggested that this species requires 2 years to develop to maturity in the Arctic Ocean. Grainger's stage frequencies given for *M. pygmaeus* from the ice island T-3 clearly show the progression of a group at an intermediate stage of development (from C4 to C5) and the persistence of this stage through July, August, and September. In the data from Tanquary Fjord the progression of an intermediate group is also evident. The persistence through August of this stage mode, however, is not clearly demonstrated. It should be noted that, even if all of the intermediate group reached adulthood during late July, it is probable that successful reproduction could not occur until the following spring. Although more extensive data are needed to confirm the seasonal cycle of *M. pygmaeus*, it is suggested that 2 years is required for successful reproduction of this species.

Most data on *Calanus* from the high arctic are characterized by a persistence of copepodite stages throughout the sampling period and a lack of definite progressions of stage modes. For example, Brodsky and Nikitin (1955) found a wide seasonal spread of copepodite stages of *Calanus* sp. in the Arctic Ocean in 1950 and 1951. Johnson (1963) also found this persistence of copepodite stages during the winter months in the Arctic Ocean. In addition, he observed no nauplii of this genus during the period sampled (from September 1957 to February 1958) which negates the possibility that breeding occurs during the winter months. Grainger (1965) remarked on the persistence of early copepodites of *C. glacialis* throughout the summer in the western high arctic. He suggested that this species takes at least 2 years to reach maturity in the Arctic Ocean. At Tanquary Fjord both *C. glacialis* and *C. hyperboreus* demonstrated an extremely slow rate of development. As a result, if the population remained as a single stage mode, breeding could occur only during occasional years. It is concluded that these two species reproduce in an opportunistic manner. The breeding cycles are not synchronized with the annual enviromental conditions but rather the animals develop slowly to maturity over a period of several years. Clearly no definitive picture can be drawn concerning the breeding cycles of these larger herbivorous zooplankters in water of such low productivity. The same may hold true for the predatory species *Sagitta elegans* and *Parathemisto abyssorum.*

ACKNOWLEDGMENTS

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REFERENCES

APOLLONIO, S. 1961. Chlorophyll content of Arctic sea ice. Arctic, **14**: 197–200.

BOGOROV, V. 1946. Zooplankton collected by the " Sedov " expedition. 1937–1939. Trudy, **3**: 356–370.

BRODSKY, K. A., AND M. N. NIKITIN. 1955. Hydrobiological work. Observational data of the scientific research drifting station of 1950–1951. Vol. 1. Sect. 4. Art. 7. 6 p. 4 Appendices. (Am. Meterol. Soc. Transl.)

CANADIAN OCEANOGRAPHIC DATA CENTER. 1966. Arctic, Hudson Bay and Hudson Strait. Data Record Ser. 4. 247 p.

CARTER, J. C. 1965. The ecology of the calanoid copepod *Pseudocalanus minutus* Krøyer in Tessiarsuk, a coastal meromictic lake of northern Labrador. Limnol. Oceanog., **10**: 345–353.

DIGBY, P. S. 1950. The biology of the small planktonic copepods of Plymouth. J. Marine Biol. Assoc., **29**: 393–438.

1954. The biology of the marine planktonic copepods of Scoresby Sound, East Greenland. J. Animal Ecol., **23**: 298–338.

FISH, C. J. 1936. The biology of *Pseudocalanus minutus* in the Gulf of Maine and the Bay of Fundy. Biol. Bull. Woods Hole, **70**: 193–216.

FONTAINE, MARION. 1955. The planktonic copepods (Calanoida, Cyclopoida, Monstrilloida) of Ungava Bay, with special reference to the biology of *Pseudocalanus minutus* and *Calanus finmarchicus.* J. Fish. Res. Bd. Canada, **12**: 858–898.

FORD, W. L., AND G. HATTERSLEY-SMITH. 1965. On the oceanography of the Nansen Sound fjord system. Arctic, **18**: 159–171.

GRAINGER, E. H. 1959. The annual oceanographic cycle at Igloolik in the Canadian Arctic. 1. The zooplankton and physical and chemical observations. J. Fish. Res. Bd. Canada, **16**: 453–501.

1965. Zooplankton from the Arctic Ocean and adjacent Canadian waters. Ibid., **22**: 543–564.

HANSON, K. J. 1961. The albedo of sea ice and ice islands in the Arctic Ocean. Arctic, **14**: 188–196.

JOHNSON, M. W. 1963. Zooplankton from the high Polar Basin. Limnol. Oceanogr., **8**: 89–102.

MARSHALL, S. M. 1949. On the biology of the small copepods in Loch Striven. J. Marine Biol. Assoc., **28**: 45–122.

MARSHALL, S. M., AND A. P. ORR. 1955. The biology of a marine copepod. Oliver and Boyd, London. 188 p.

MCLAREN, I. A. 1963. The effect of temperature on growth of zooplankton and the adaptive value of vertical migration. J. Fish. Res. Bd. Canada, **20**: 685–727.

1965. Some relationships between temperature and egg size, body size, development rate, and fecundity, of the copepod *Pseudocalanus.* Limnol. Oceanogr., **10**: 528–538.

OSTVEDT, O. J. 1955. Zooplankton investigations from Weather Ship ' M ' in the Norwegian sea, 1948–49. Hvalradets Skrifter Norske Videnskaps-Akad. Oslo, **40**: 1–93.

RUUD, J. T. 1929. On the biology of copepods off More. Rappt. Conseil Intern. Exploration Mer, **56**: 5–84.

USSING, H. H. 1938. The biology of some important planktonic animals in the fjords of east Greenland. Medd. Groenland, **100**: 1–108.

WIBORG, K. F. 1944. The production of zooplankton in a landlocked fjord the Nordasuatn near Bergen. Rept. Norweg. Fish. Marine Invest., **7**: 1–83.

1954. Investigations on zooplankton in coastal and offshore waters off western and Northwestern Norway, with specal reference to the copepods. Ibid., **11**: 1–246.

Feeding and Migration Patterns of the Pink Shrimp, *Pandalus montagui*, in the Estuary of the River Crouch, Essex, England[1]

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ABSTRACT

Feeding of the pink shrimp, *Pandalus montagui*, took place mainly in daylight at low slack water. There was relatively little feeding at low slack water at night, and almost no feeding at other times. Sand grains were ingested with the food and it was possible to identify areas where feeding could have occurred by comparing grain-size distributions of material from the stomachs with those of the bottom deposits. It was possible to show, by fishing in selected areas and by considering patterns of tidal flow, that feeding was restricted to a small area near the mouth of the River Crouch, and that *P. montagui* migrated from this area with the flood tide and returned to it on the ebb.

INTRODUCTION

On the east coast of England the pink shrimp, *Pandalus montagui*, is locally abundant and is fished commercially in the area around the estuary of the River Thames and in the Wash. Commercial quantities are found in inshore waters from March until October. During this time the shrimp appear to make short and relatively rapid migrations, and may suddenly arrive at or depart from very localized areas. It has been the accepted practice among commercial fishermen to search with a small hand dredge for the polychaete worm *Sabellaria spinulosa* and then trawl for shrimp in areas where this was found. When available, *S. spinulosa* is believed to be a major item in the diet of *P. montagui*, and short migrations might be made to search for this or other food organisms. *Sabellaria spinulosa* forms a colonial honeycomb structure, often of considerable size, and large quantities of this apparently desirable food organism can occur in relatively small areas. Studies by Mistakidis (1957) included work on types of food taken by *P. montagui* in the River Crouch, and in this paper further work is described relating feeding habits to locality, time of day, and state of tide.

The major features of the hydrography of the estuary of the River Crouch have been described by Knight-Jones (1952) and Waugh (1957). There is only a small amount of freshwater runoff, particularly in summer. The drainage basin of the river is relatively small and there are no tributaries of any consequence.

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The disproportion in size between the estuary and the river has arisen because the estuary is a former outlet of the River Thames (Wooldridge and Linton, 1955). Salinity is uniformly high (32–36 ‰) except for short periods during winter when in the upper reaches it can fall to 20 ‰. Tidal currents are relatively strong (80 cm sec^{-1} or 1.6 knots), but there is relatively little mixing with the open sea. Water carried out of the estuary by the ebb tide is contained within a tidal channel between sandbanks and is carried back again by the flood tide.

The distribution of bottom sediment and the variation of grain-size characteristics both within the estuary and offshore were known (R. W. Sheldon, unpublished data), so that when sand with definite grain-size characteristics was discovered in the stomach of *P. montagui* it was possible to show that this could only have been ingested in certain areas. Experiments were carried out to investigate the pattern of ingestion and egestion of food and sand and to find the localities where feeding could have occurred.

METHODS

Shrimps were caught with a 4-m or 6-m beam trawl, and all hauls were for 30 min. A large sample was taken randomly from the catch, not more than 5 min after the trawl was hauled inboard, and preserved in formalin. Sub-samples of 100 were used for analyses of stomach contents.

Two series of samples were taken. In the first series hauls were made at hourly intervals for 24 hr during November 28–29, 1963. The other series were taken at the same time each day (0900) during March 15–April 11, 1965. All samples were from the same locality (Fig. 1).

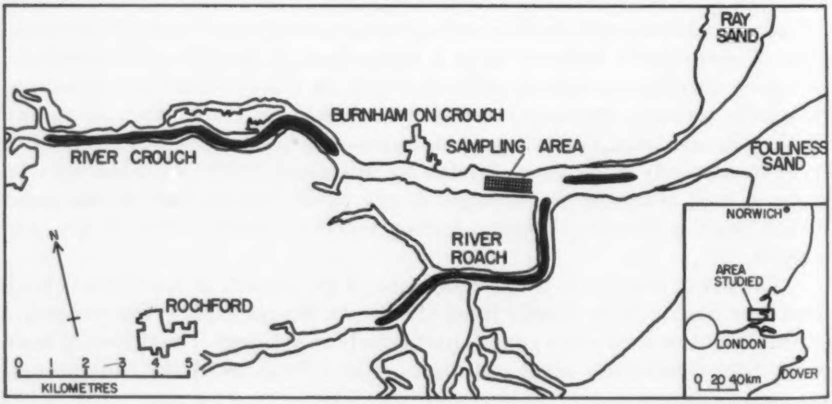

FIG. 1. Distribution of sediment in the estuaries of the River Crouch and the River Roach. The sampling area for shrimps is shown hatched. Areas with modal grain-size of 70–80μ are shown as solid black.

The stomach contents of the 1963 samples were examined with a dissecting microscope and classified into four groups: (*a*) complete organisms or undigested fragments of recognisable species assumed to be digestible; (*b*) amorphous, partly digested material; (*c*) structures assumed to be indigestible, e.g. shell fragments; (*d*) sand grains. Presence or absence of each group was noted for each individual, and frequency of occurrence was calculated for each sample.

The bottom sediments of the estuary were sampled with a 0.1 m^2 Van Veen grab, and only the top 1 cm of an undisturbed part of the sample was collected. The sediments are made up of two distinct populations of particles. There is a fine sand and silt fraction with grain diameters larger than about 25 μ, and a silt and clay fraction with diameters less than this (Fig. 2). The fine sand and silt fraction was separated from the sediment by screening onto a 37-μ standard sieve. The grain-size distribution of the material retained by the sieve was log-normal (Fig. 2). and the mode was taken as a measure of average grain size.

The sediments were prepared for grain-size analysis in the following way. A small sample (about 0.2 g) was dispersed by simmering for about 3 hr on a hotplate (110 C) with 3% hydrogen peroxide, and then screened. The material retained consisted of sand grains, relatively large organic fragments which had not been fully digested by the hydrogen peroxide, and shell fragments. The shell fragments, which were not abundant, were removed with hydrochloric acid and the organic matter was removed by ignition at 550 C for 45 min.

Sand from the stomachs of *P. montagui* was treated in a similar way, but the sequence of operations was slightly different. One hundred stomachs

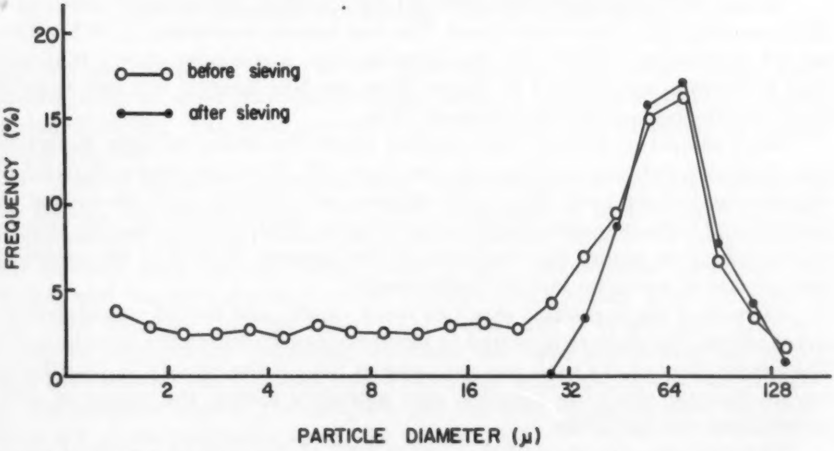

FIG. 2. Grain-size distribution of an intertidal sediment from the middle reaches of the estuary of the River Crouch. The solid circles show the effect of sieving through a mesh of 37 μ. This particular type of sediment was selected as an example because of the relatively large fine fraction. Sediment from the feeding and sampling areas is similar but there is less fine material and the effect of sieving is less noticeable.

were dissected out and ignited for 45 min at 550 C in a silica crucible. After cooling, shell fragments were removed with hydrochloric acid. Dispersion of the sample, which at this stage consisted of sand grains and ash, was brought about by ultrasonic vibration, and the ash wash removed by washing onto a 37-μ sieve.

After this treatment, samples of both bottom sediment and material from the stomach of *P. montagui* consisted only of sand grains. Grain sizes were measured with a Coulter counter (Sheldon and Parsons, MS, 1966) using a 3% NaCl solution of 50% glycerol and water as electrolyte. Before any measurements were made, the samples were put in an ultrasonic bath for a few minutes to thoroughly disperse the grains in the electrolyte. The coefficient of variation of modal diameter of the grain-size distributions was 2%.

EGESTION OF SAND

Before observations on sand from the stomach of *P. montagui* could be of value, either for identifying areas where feeding occurred or for investigating feeding behaviour, it was necessary to find out how long sand remained with the shrimp. Two experiments were carried out during May 12–14, 1965 to determine this.

Shrimps were caught during the middle part of the flood tide. A sample was taken and preserved just after the catch had been removed from the trawl. The remaining shrimps (about 1000) were then conveyed live to the laboratory, arriving there about 1 hr after being caught. They were put in a smooth concrete tank (capacity 1000 litres) filled with clean sea water.

In the first experiment they were left for a further hour to settle down and then samples were taken every hour. The last sample was taken at 10 hr. The second experiment, carried out the following day, was similar except that the time between samples was 3 hr. After 15 hr the 3-hr interval was not strictly kept, and the last sample was taken at 29 hr.

Each sample of shrimps was weighed before the stomachs were dissected out. Variation between samples was less than 1%. We have shown that large shrimps carry more sand than small shrimps and that the amount carried is proportional to body weight (Sheldon and Warren, 1966), so that this precaution was necessary to ensure that variation in the amount of sand in the samples was not due to variation in total body weight.

In spite of the inevitable shock of being caught and moved to unfamiliar surroundings, the shrimps appeared to behave normally during the experiments. The temperature of the tank was the same as that of the estuary so that there was no thermal shock on transfer, and mortality during the course of the experiments was negligible.

These experiments showed that about 50% of the original weight of sand was present after about 8–10 hr, 20% after 16 hr, and 10% after 27 hr (Fig. 3). There were no significant changes in grain-size distribution during the experiments, indicating that the grains were egested non-selectively. If a rate of

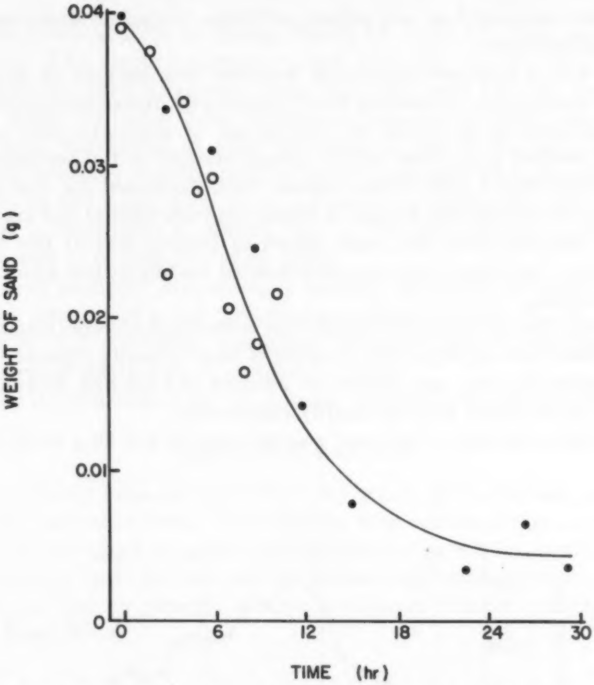

FIG. 3. Variation with time of the weight of sand from 100 stomachs of *P. montagui* kept in a tank. The line is fitted by eye.

egestion similar to that found in the experiments occurred under natural conditions, then accurate grain-size analyses could be made at least 12 hr after a period of feeding. This time interval could be considerably increased by taking larger samples.

Most of the sand grains and other large indigestible particles seemed to be egested by regurgitation. The lower intestines of several large shrimps contained only green and brown amorphous material, yet some of the objects in the stomach were relatively large (about 2 mm) and included operculae, fish scales, and complete shells of small molluscs. Such things were not seen in the lower intestine (internal diam. 0.5–1.0 mm) and it is probable that they would be regurgitated. Additional evidence that sand grains were not passed into the intestine was obtained from a sample in which the bodies were divided at the posterior edge of the carapace and the sand extracted from each half. Only 6% of the total sand was in the tails.

FEEDING PATTERN

Two series of sub-samples were taken from the material collected during November 28–29, 1963, and the stomachs were dissected out. Individual stomach contents were examined in one series, and from the other series sand was extracted quantitatively.

There was a positive correlation between the amount of sand and the percentage of stomachs containing fresh, potentially digestible material (Fig. 4). Both the amount of sand and the percentage of stomachs with recently ingested food reached maximum values during daylight in the period of low slack water and early flood tide. These values then decreased for the next 15 hr, with a slight rise in the percentage of stomachs with food at low slack water at night. This suggests that the main period of feeding was at low slack water during the day, and that there was only limited feeding at low water during the hours of darkness.

There was a rough negative correlation between the proportion of stomachs with easily observed sand and the amount of sand actually present. The reason for this is probably that only when the amount of food had decreased (by digestion) did sand grains become readily discernable.

The modal grain-size of the sand was between 70 and 80 μ in all the samples analysed.

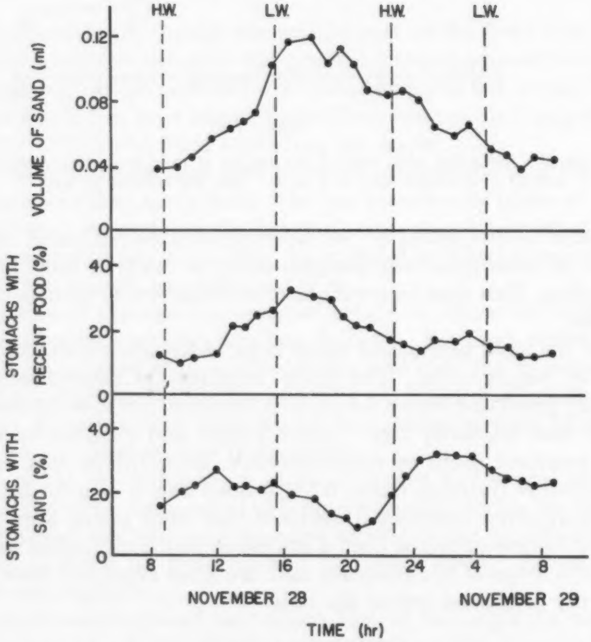

FIG. 4. Variation in food and sand in 100 stomachs of *P. montagui* during November 28–29, 1963. H.W. = high water; L.W. = low water (data smoothed by taking running means of 3 values).

Samples taken at 0900 hr during March 15–April 11, 1965, were analysed for sand only. The amount present varied from day to day (Fig. 5). There were two periods when relatively large amounts were carried, spaced at an interval of about 14 days. This suggests an association with the tides. The data have been rearranged to show the state of tide at the time of sampling (Fig. 6), and here the relationship is quite apparent. The greatest amounts of sand were carried near the time of low water or during the first part of the flood tide. It is reasonable to believe that in this case also the main period of feeding occurred at or near low slack water.

There were probably differences in feeding behaviour between March and April, as the amount of sand carried was greater during March. This could indicate either that more food was taken during March, or that in April less sand was ingested relative to the amount of food.

The modal grain-size of all the samples was between 70 and 80 μ.

MIGRATION PATTERN

Two significant facts emerged from the study of the feeding pattern. The first was that sediment from the stomachs was not the same as the bottom sediment of the sampling area; the modal grain-size from the stomachs (70–80 μ) being significantly less than that of the bottom sediments (greater than 90 μ). The second was that, in general, maximum amounts of sand were carried on the first of the flood tide.

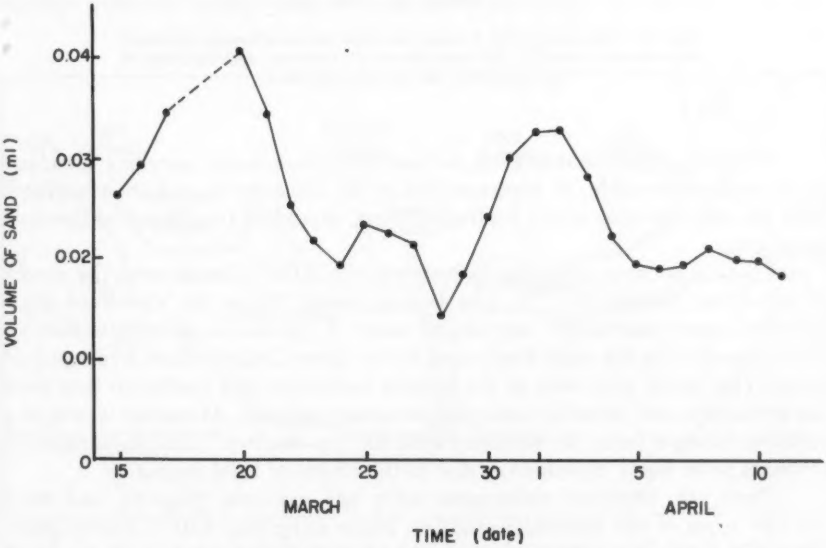

FIG. 5. The volume of sand from samples of 100 stomachs of *P. montagui* taken at 0900 hr each day during March 15–April 11, 1965 (data smoothed by taking running means of 3 values).

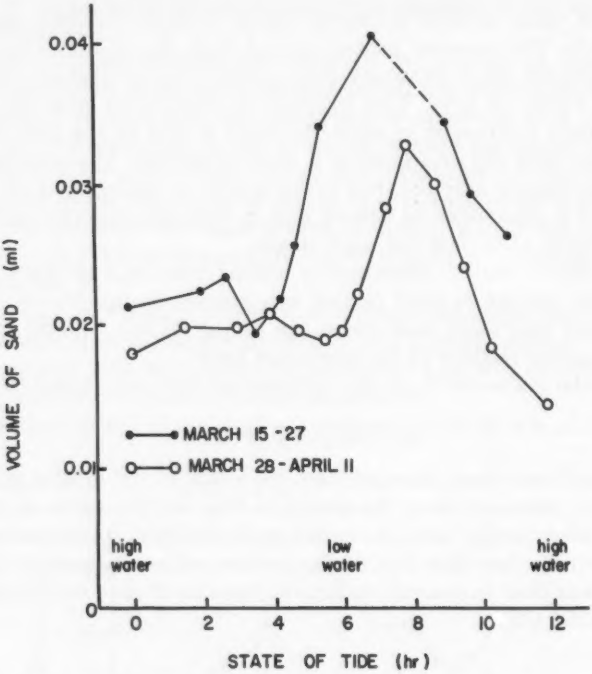

FIG. 6. Data from Fig. 5 recast to show the relationship between the volume of sand in 100 stomachs of *P. montagui* and the state of the tide when the sample was taken.

These suggested that feeding did not take place in the sampling area, and if, as seems reasonable, *P. montagui* fed at low slack water and then migrated with the tide, the area where feeding occurred should lie to seaward of the sampling area.

There is an area with fine sediment in the River Crouch near the mouth of the River Roach (Fig. 1). The bottom fauna, taken by Van Veen grab, included many segmented worms and some *S. spinulosa* and *Harmothöe* sp. This seemed to be the most likely area in the River Crouch where feeding could occur. The modal grain-size of the bottom sediments was similar to that from the stomachs, and suitable food organisms were present. Moreover it was at a suitable distance from the sampling area for the observed changes in stomach contents to be easily explained as due to the effects of tidal migration.

There are, however, other areas with fine sediment (Fig. 1), and there are also parts of the intertidal mudflats where sediments with a modal grain-size of 70–80 μ occur. Trawl hauls were made in these and other areas, both at high and low water, to find out where high concentrations of shrimp occurred.

No shrimps were caught on the intertidal mudflats near to the sampling area (observations made only at high water). Small numbers (average 20 per haul) were taken both from the mouth of the estuary and above Burnham on Crouch. Moderate numbers were caught in the River Crouch at and just above the mouth of the River Roach (more than 200 per haul) and the largest catches were made at low water just to seaward of the mouth of the River Roach (more than 500 per haul). There can be little doubt that the area in which maximum catches were made was the main feeding ground for shrimps in the River Crouch (Fig. 7).

The pattern of feeding and migration could be interpreted as follows. The main period of feeding would occur at low slack water during daylight. Shrimps would move away from the feeding area and into the sampling area with the flood tide. No feeding would take place at high slack water and shrimps would move back to the feeding area with the ebb tide (Fig. 7). If the return to the feeding area was during darkness only a small amount of feeding would take place.

Some observations were made in the River Roach. Here also there are areas where the modal grain-size of the bottom sediments is similar to that of the sand from the stomachs of *P. montagui* taken in the River Crouch (Fig. 1). The composition of the bottom fauna is such that suitable food for shrimps could be present (Mistakidis, 1951), and good catches of shrimp were taken (Fig. 7). It is possible that feeding and migration patterns similar to those of the River Crouch were followed in the River Roach, and some movement of shrimps between the two estuaries could have occurred.

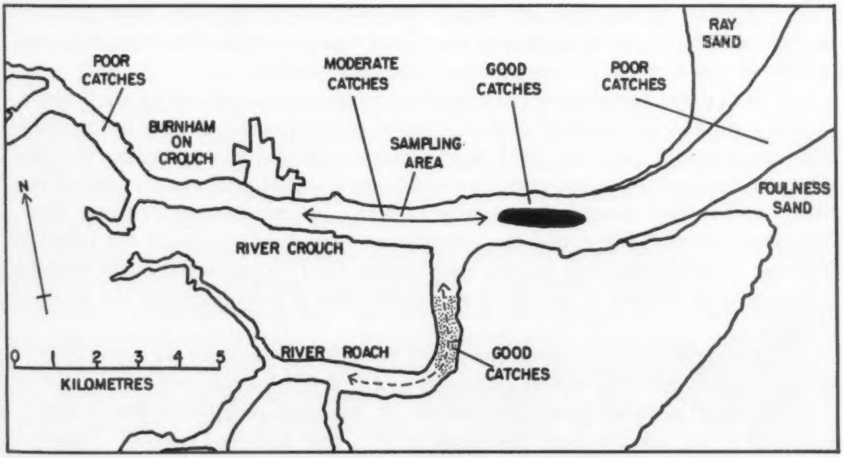

FIG. 7. Feeding area of *P. montagui* in the River Crouch (shown as solid black) and possible feeding area in the River Roach (shown stippled) with the suggested course of diurnal tidal migration (arrows).

DISCUSSION

Shrimps are often found associated with particular types of bottom sediment. Williams (1958) has shown that when three species of penaeid shrimp were allowed a free choice of substrate, two species preferred soft muddy sediment, but the third preferred shell sand. This agreed with field observations. He also noted that sand was found in the stomach and suggested that the food content of the sediments could have influenced the choice of substrate.

Our results demonstrated that *P. montagui* was very selective in choice of substrate and that this might be a result of food-seeking activity. It is possible that it was the principal food organisms which were selective and the distribution of these was followed by that of *P. montagui*. Whatever the underlying reason might be, *P. montagui* was able to select the preferred substrate with considerable precision. Feeding took place in an area with sediment with a modal grain-size less than 80 μ but not in an area with modal grain-size greater than 90 μ. No work was done specifically on the fine fraction of the sediments but it was known that a decrease in modal grain-size was associated with an increase in the proportion of fine material. Sediments with a relatively large proportion of fine material were also more cohesive than those with less fine material (R. W. Sheldon, unpublished data). Sanders (1958) has shown that changes in the proportion of fine material in sediments can have considerable effects on the fauna. It is possible that in the River Crouch, although the change in modal grain-size is slight, the change in proportion of fine material could considerably affect sedimentary properties of importance to small benthos (e.g. cohesion, aeration), and hence to the food of shrimps.

The apparent lack of feeding activity in the sampling area was not due to any repellent property peculiar to the area. Several fish species were caught with sediment in the stomach similar to the bottom deposits (Sheldon and Warren, 1966). There is no doubt that food was present in the sampling area but it did not seem to be acceptable to the pink shrimp.

The distinct correlation between the proportion of stomachs with recognisable food and the amount of sand carried is noteworthy. It was unfortunate that the measure used for food (undigested, identifiable fragments) was not very satisfactory. It is not possible to express this estimate clearly and objectively, and further work is being done to find a less subjective measure. However, there seems little doubt that, on the average, food and sand are taken in constant proportions. This is very convenient, for it is much easier to measure the amount of sand in a sample of stomachs than it is to estimate the amount of food.

The migration of *P. montagui* could be followed because we were able to measure accurately the modal grain-size both of the bottom sediments and of the very small samples from the stomach. It has been possible to detect movements of other species over distances from a few hundred metres (*Carcinus maenas*) to more than 20 km (*Raia batis*) (Sheldon and Warren, 1966). In areas where the bottom sediments have either uniform properties or complex patterns of distribution, the techniques described in this paper could be modified. For instance, it might be possible to lay down a small amount of distinctive mineral in a suspected feeding area and then search for this in the stomachs of the animals being studied. It should be possible, with a suitable mineral, to detect a single grain in a sample of stomach contents. There are obvious difficulties in this type of approach, but it has the advantage over conventional tagging methods that there would not be any interference with the normal life of the animal.

There are probably two basic reasons why the shrimps fed only in daylight at low slack water. The first is that their density (1.04 g/ml) is near to that of sea water, so that in the water their inertia would be very small. It is unlikely that they would be able to resist the full force of the tidal current; they would therefore tend to move with the tide, though not necessarily at the same speed as the tidal current. This would limit their most effective feeding to the periods of slack water. The second is that feeding activity appears to be controlled by light, and at low slack water during daylight, illumination of the bottom is at a maximum. The depth of water is at a minimum and the turbidity is less than at other times during the tidal cycle (R. W. Sheldon, unpublished data). These two factors, coupled with that of food availability, could determine the pattern of feeding.

It was not possible to decide, on the basis of grain-size alone, whether shrimps caught in the sampling area fed in the River Crouch or in the River Roach. However, if the pattern of tidal flow is taken into consideration it is clear that most of the shrimps fed in the River Crouch. In order to move from the River Roach and be taken in the sampling area, shrimps would have to leave the River Roach on the last of the ebb tide, then feed and remain in the River Crouch during the flood tide. This kind of movement could occur but it is probable that only relatively small numbers would be affected at any one time. A second population may have fed and moved in the River Roach (Fig. 7) but we made no observations of this.

It is possible that the pattern of feeding and migration which we have described persists from year to year, as the results of November 1963 are surprisingly similar to those of March–April 1965. There is a seasonal movement to and from the estuary (Mistakidis, 1957), but the pattern of behaviour when in the estuary might remain constant.

Finally, we would emphasise that the conclusions we reached were based on observations of average quantities and conditions. For instance, in any one sample some stomachs were full and some were empty. Variation between samples was reduced by taking 100 stomachs together. Also, serial observations were smoothed by taking running means. Not all the shrimps congregated in the feeding area at low water, and it was possible to find small numbers of shrimp in many parts of the estuary at all states of tide. It will be clear therefore that although a large proportion of the shrimp population followed the patterns of behaviour we have described, it was possible at any time to find some shrimps which apparently did not.

ACKNOWLEDGMENTS

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REFERENCES

KNIGHT-JONES, E. W. 1952. Reproduction of oysters in the Rivers Crouch and Roach, Essex, during 1947, 1948 and 1949. Fish. Invest. London, Ser. 2, **18**(2).

MISTAKIDIS, M. N. 1951. Quantitative studies of the bottom fauna of Essex oyster grounds. Ibid., **17**(6).
1957. The biology of *Pandalus montagui* Leach. Ibid., **21**(4).

SANDERS, H. L. 1958. Benthic studies in Buzzards Bay 1. Animal-sediment relationships. Limnol. Oceanogr., **3**: 245–258.

SHELDON, R. W., AND T. R. PARSONS. MS, 1966. On some applications of the Coulter counter to marine research. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanogr. Limnol.). No. 214. 36 p.

SHELDON, R. W., AND P. J. WARREN. 1966. Transport of sediment by crustaceans and fish. Nature, **210**: 1171–1172.

WAUGH, G. D. 1957. Oyster production in the Rivers Crouch and Roach, Essex, from 1950 to 1954. Fish. Invest. London, Ser. 2, **21**(1).

WILLIAMS, A. B. 1958. Substrates as a factor in shrimp distribution. Limnol. Oceanogr., **3**: 283–290.

WOOLDRIDGE, S. W., AND O. L. LINTON. 1955. Structure, surface and drainage in southeast England. George Phillip, London. 176 p.

Ekman Transport and Zooplankton Concentration in the North Pacific Ocean[1]

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ABSTRACT

Annual concentrations of zooplankton off California varied directly, and concentrations in the western Bering Sea varied indirectly with calculated southerly components of Ekman transport in the previous year at 50°N,140°W. This point is in the area in which surface subarctic water and the deeper currents divide north and south. The underlying geostrophic flow requires approximately 1 year to reach each downstream sampling area. Summer concentrations of zooplankton at Ocean Station P, 50°N,145°W, varied with southerly and westerly components at 50°N,160°W during the previous winter. Fifty to sixty per cent of the variance of the annual concentration in zooplankton appears to be due to advection of surface water.

INTRODUCTION

ADVECTION is recognized as a major influence on the physical characteristics of both the atmosphere and the ocean in the northeast Pacific region (Namias, 1953; Eber and Sette, 1959). Sette and Isaacs (1960) pointed out that the evidence presented by the members of their symposium clearly and unequivocally showed that locally observed changes in ocean conditions, marine fauna, fisheries success, weather, etc. are often the result of processes acting over vast areas of the Pacific Ocean. They suggested that an understanding of local biological phenomena may be dependent on studies of wider oceanic areas, and that the aberrant conditions of 1957 and 1958 (in the Pacific Ocean) appear to have been largely related to the response of the water to the winds.

Sverdrup et al. (1942) indicated that it is normal for animals to be transported great distances in the main oceanic currents. Some recent examples are: gastropod larvae, 3000 miles from Florida to the Azores (Scheltema, 1966); sterile *Poeobius*, 2000 miles from 20°N,120°W to 0°N,90°W (McGowan, 1960); cod eggs and larvae, 600 miles from the Lofoten Islands to Spitzbergen (Corlett, 1964); the subarctic *Euphausia pacifica* into southern California waters (Reid et al., 1958).

In addition to the direct transport of animals, Williamson (1961) analyzed the contributions of vertical mixing, surface temperature, and ocean advection to the abundance of plankton in the North Sea. Sette (1957) described the processes whereby the divergence of the South Equatorial Current along the equator brings nutrient-rich water to the surface which then moves westward. Upwelling is most intense about longitude 120°W. The maximum standing

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crops of zooplankton were found 1800 miles downstream between 150°W and 160°W (Schaefer, 1961). Eber and Sette (1959) and Corlett (1964) have given statistical evidence that annual variations in winds effect water movement and the drift of animals, and that barometric pressure records are useful tools for the study of animal populations in the sea.

Finally, Reid (1962) has collated the data on circulation, inorganic phosphate-phosphorus in the upper levels, and zooplankton volumes for the Pacific Ocean and found correspondence between all three quantities over the entire Pacific. He showed that the data were consistent with the concept of the maintenance of plankton populations by systems rotating in fixed positions as proposed earlier (Sverdrup et al., 1942, and others). Further he presented graphic evidence that in years of low temperature (and presumably greater influx of subarctic water) zooplankton volumes off lower California were highest.

In the light of the above studies, it is apparent that either singly or together, nutrients and animals are transported long distances in the ocean and that the centres of zooplankton abundance are likely to be found downstream of points of upwelling.

The present paper examines the possibility that annual densities of zooplankton in the North Pacific may be strongly influenced by conditions far "upstream" at the diversion point of the West Wind Drift, near its eastern boundary.

DATA USED

Annual zooplankton volumes off California (Thrailkill, 1963), in the western Bering Sea (Fisheries Agency of Japan, 1963), and at 50°N,145°W (LeBrasseur, 1965) are available for comparison with mass transport in the North Pacific Ocean (Fofonoff, 1962; Fofonoff and Dobson, MS, 1963). Data for the area off California are available only for the months of February to August and those for the western Bering Sea are available for only June and July. Data for February to August were taken at P for comparison with the California data. This period covers the time of major annual variation of zooplankton.

Froese (1963) has re-written the mass transport computation program for the IBM 1620 computer. Mr A. G. Fowler of the Computing Centre at the University of British Columbia, Vancouver, B.C., modified this program for the IBM 7040 computer. Atmospheric pressure data cards were made available through the courtesy of the Extended Forecast Division of the United States Weather Bureau so that the series of Ekman transport computations were extended to cover the years from 1946 to 1965 (Wickett, MS, 1966).

The Ekman transport, which is movement of water in approximately the upper 100 m due to wind stress, was computed as meridional (north +; south −) components and as zonal (east +; west −) components for alternating 5° of latitude and longitude. Monthly mean sea-level pressures at seven points were used in computing the transport at each grid point (Fig. 1). Fofonoff (1962) discussed the inherent difficulties of using monthly mean pressure as the basic

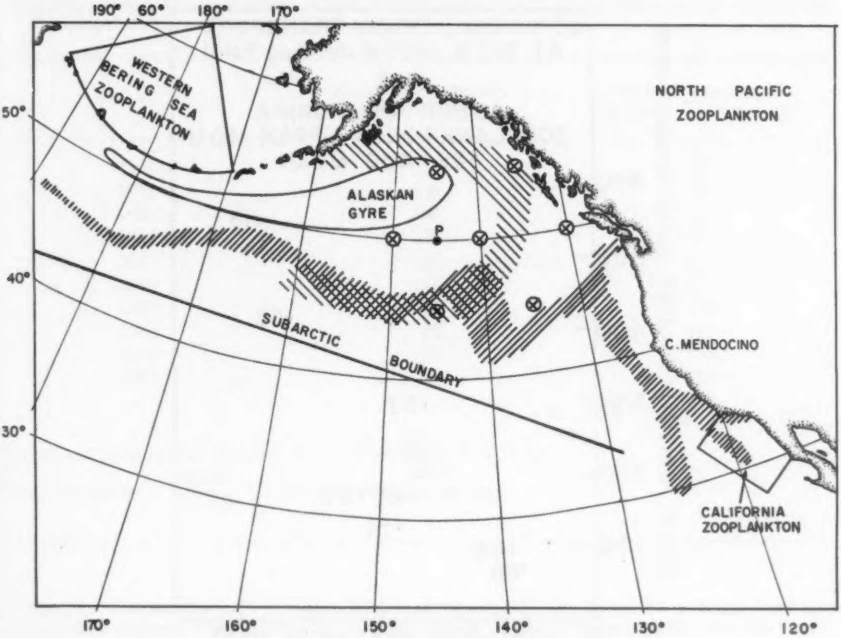
190°
60°
180°
170°
50°
40°
30°
WESTERN BERING SEA ZOOPLANKTON
NORTH PACIFIC ZOOPLANKTON
ALASKAN GYRE
P
SUBARCTIC BOUNDARY
C. MENDOCINO
CALIFORNIA ZOOPLANKTON
170°
160°
150°
140°
130°
120°

FIG. 1. Map of the Northeast Pacific Ocean showing the seven points used in computing Ekman transport at 50°N,140°W and the concentration of zooplankton according to Reid (1962) (diagonals: down to left) and according to Parsons et al. (1966) (diagonals: down to right).

input rather than using the monthly mean of transport calculated for each day. Two arguments justifying its use may be made. The changes in both environment and animal density are great, and several months' values are averaged (or totalled) for the basic measures.

CALIFORNIA ZOOPLANKTON

February to August zooplankton volumes off lower California for the years 1951 to 1960 varied directly with the meridional component (southerly) for the months of January to August at 50°N,130°W; 50°N,140°W; 50°N,150°W; 50°N,160°W; and at 45°N,135°W in the previous year. The closest agreement was noted at 50°N,140°W: $r = 0.8$ (Fig. 2). Agreement was fairly high, $r = 0.7$, along 50°N and at 45°N,135°W but not to the north or south. There was no agreement between zooplankton and Ekman transport in the same year: $r = 0.23$. The annual variations in the meridional component of total transport at 50°N,140°W were unrelated to zooplankton variations in the same period. The total transport is an order of magnitude greater than the surface transport (Ekman transport) and is the sum of the transports by surface and underlying geostrophic flows. The hypothesis presented below assumes that *annual varia-*

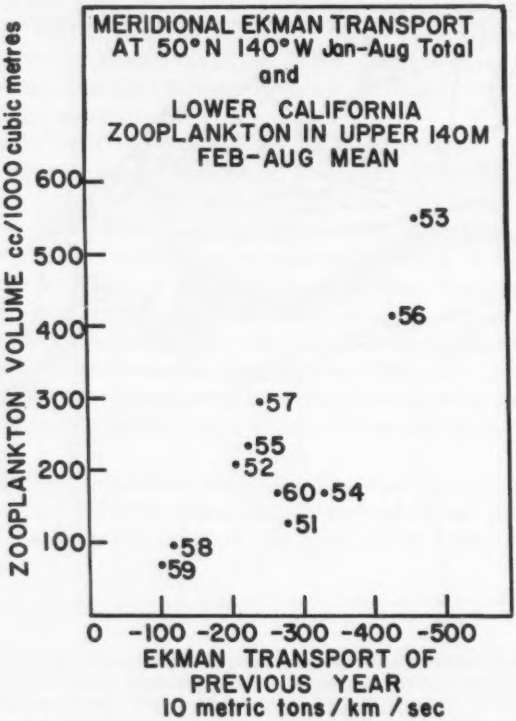

FIG. 2. Plot of zooplankton concentration off lower California and the southerly component of Ekman transport 1200 miles upstream at 50°N,140°W in the previous years.

tions in flow at depth can be neglected, and that the flow in the upper 100 m or so, relative to the flow in the deep layers, is the important factor.

BERING SEA ZOOPLANKTON

A second series of annual zooplankton abundance is given for the years 1955 to 1963 in the western Bering Sea by the Fisheries Agency of Japan (1964) (Table II). Figure 34 of their paper gives the average wet weight of zooplankton collected in June and July. They say that it is essential to take into account the extent of the westward extension of the Alaskan Stream when considering the abundance of zooplankton in the northwest Pacific. The Fofonoff zonal components of surface transport over the Alaskan Stream and of integrated total transport in the Gulf of Alaska were not found to be closely related to the western Pacific zooplankton abundance. Southerly meridional surface components at 50°N,140°W in the previous year were related inversely and signif-

TABLE I. California zooplankton density in the upper 150 m during February to August and meridional Ekman transport at 50°N,140°W for January to August in the previous year.

	Zooplankton density (*cc/1000 m^3*)	Southward transport (*10 metric T/km per sec*)
1951	127	278
1952	210	205
1953	550	461
1954	170	327
1955	235	224
1956	415	428
1957	295	238
1958	95	119
1959	70	105
1960	170	264

$r = 0.84$; (5% = .632; 1% = .765)

Per cent of zooplankton variance due to transport is $r^2 = 70\%$

Mean transport $= \frac{26490}{10 \times 8} = 330$ metric T /km per sec

TABLE II. Western Bering Sea zooplankton average weight in the upper 150 m during June and July and meridional Ekman transport at 50°N,140°W for April to June in the previous year.

	Wet weight zooplankton (*g/1000 m^3*)	Southward transport (*10 metric T/km per sec*)
1955	480	52
1956	240	172
1957	250	78
1958	500	21
1959	437	28
1960	335	47
1961	275	83
1962	370	62
1963	375	86

$r = -0.75$; (5% = .666; 1% = .798)

Per cent of zooplankton variance due to transport is $r^2 = 56\%$

icantly to the western Bering Sea figures: $r = -0.75$. When the southerly meridional Ekman components for the months of April, May, and June are small, a greater proportion of the surface water from the West Wind Drift moves northward into the Gulf of Alaska current system. Part of this water is drawn into the anticlockwise gyre and part proceeds to the west in the Alaskan Stream.

The averaging of the geotrophic flows of 5 miles per day along the coast of southeast Alaska, 10 miles per day along the Aleutian Peninsula, and 2 miles per day around the Bering Sea Gyre gives a value of 6 miles per day for the whole course. This is in reasonable accord with the 2200 miles around the Alaskan Gyre from 50°N,140°W to the Bering Sea Gyre being travelled in 360 days, or the 2700 miles to the Western Subarctic Gyre at approximately 45°N,165° E in the same time.

The water reaching the Bering Sea in certain years is comparatively nutrient poor, having drifted across the Pacific from west to east without becoming enriched by upwelling or by other processes along the North American coast. A strong southerly meridional component of Ekman transport would tend to drive much of the water from the region of West Wind Drift divergence toward the California coast. This would weaken the northward flow into the Gulf of Alaska and the input of enriched water into the Alaskan Stream. By the above estimation of time for the water to reach the Bering Sea Gyre, the effect of the nutrient-poor water on zooplankton in the western Bering Sea would be noted a year later.

Lessened southerly Ekman transport in 1957, 1958, 1963 can be seen in Fig. 3. Components taken at 55°N,145°W (not presented here) show above-average *northerly* components in the above years, when a more northerly set of drift bottles was noted (Dodimead and Hollister, 1958; Favorite, 1965; Favorite et al., 1965).

Transport for the months of May, June, and July (and the December to March grouping used in the next section) was set and tabulated, as the most significant period for winds in terms of surface mixing, on the advice of physical oceanographers before the present study was made. On the basis of the California zooplankton study, which indicated "upstream" influences, only the one surface transport relation was tested. Total transports were examined later because variations in geostrophic flow are usually considered to play the major role in ocean regimens.

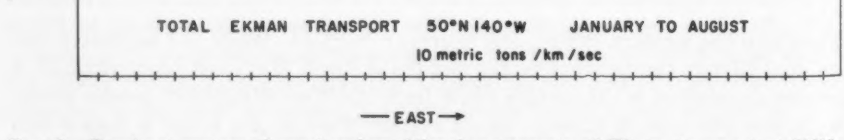

FIG. 3. Resultant vectors of zonal and meridional components of Ekman transport at 50°N, 140°W, totalled for the months of January to August. Note the pulsing to the south.

STATION P ZOOPLANKTON

A third series of annual zooplankton abundance was given for the years 1957 to 1964 at Weathership Station P, 50°N,145°W, by LeBrasseur (1965). McAllister (1961) presented a detailed study of the 1957 and 1958 data at this position. The mean zooplankton densities from February to August found each year are significantly and inversely related to the eastward zonal Ekman transport at 50°N,160°W, 500 miles to the west, for the preceding months of December through March: $r = -0.74$. The southward meridional components for the same period are directly but not significantly related: $r = 0.5$.

The multiple correlation coefficient between zooplankton and both zonal and meridional components is statistically significant: $R = 0.837$ (Table III).

The average surface salinity at Station P (Robertson et al., 1965; unpublished data) for the period February to August in the years 1958 to 1964 is significantly related to the average zooplankton abundance for the same period: $r = .793$: 5% = .707 (Table III).

A long series of chemical analyses of sea water at P is not available. Nitrite values for April, which is the month of zooplankton increase from the winter low, in the years 1957 to 1960 follow the same trend as zooplankton abundance. Present nutrient studies will bring definite conclusions in place of the present hypothesis.

TABLE III. Station P, 50°N,145°W, mean zooplankton density in the upper 150 m and mean surface salinity during February to August and meridional and zonal Ekman transport at 50°N, 160°W for December to March of the preceding winter.

	Zooplankton wet weight (*g/1000 m^3*)	Surface salinity (‰)	Zonal transport + eastward (*10 metric T/km per sec*)	Meridional transport −southward; + northward (*10 metric T/km per sec*)
(1956)			(−60)	(−99)
1957	474	32.66	146	−12
1958	1259	32.83	20	−174
1959	1199	32.90	20	−45
1960	1203	32.78	83	−139
1961	726	32.81	25	−20
1962	562	32.57	62	15
1963	260	32.55	123	127
1964	385	32.73	154	−148

Zooplankton vs. transport	5%	1%
r (zonal) = −0.74	0.707	0.834
r (merid) = 0.52	0.707	0.834
R = 0.837	0.836	.917

Zooplankton vs. salinity	5%	1%
r = 0.79	0.707	0.834

Transport components taken at 50°N,150°W have a relation similar to those at 50°N,160°W, but are not statistically significant: $R = 0.73$. The strong southerly components at this point during the winters preceding 1958, 1959, and 1960 are interesting. Zooplankton crops were high, summer salinity was high, and temperature was low in these years. Because meridional components are related to vertical transport, it appears that in the above years, large amounts of nutrients were introduced into the Gulf of Alaska along with cooler, more saline water from the deeper layers.

The upwelled water is transported eastward out beyond the edge of the gyre. During this outward transport, phytoplankton are produced and zooplankton graze and grow. This process results in maximum zooplankton density being reached in the region at the edge of the flow around the gyre (Fig. 1). The zooplankton maximum is assumed to be closer to P when the eastward zonal components are smallest.

Favorite (1964) presented evidence of recirculation of low-salinity surface water from the Alaskan Stream into the Gulf of Alaska at longitude 160°W during 1959.

McAllister (1961) showed that the maximum concentration of zooplankton lay to the west of P in the summer of 1956. The zonal components were *westward* in the preceding winter, in contrast to those of the following winters discussed here (Table III).

Tabata (1965) stated that the geostrophic currents in the vicinity of Station P are generally directed into the first quadrant, temperature and salinity changes at P are partly due to Ekman transport, and that salinity increases to the northwest of P. The dynamic environment he described is accompanied by equally dynamic biological processes.

The present data suggest strongly that, although conditions *in* the Gulf of Alaska may vary from year to year, transport out of the Gulf is the most important factor influencing zooplankton downstream.

SUGGESTED MECHANISM

The mechanism is essentially that presented by Doe (1955), by Redi (1960, 1962), and by Dodimead et al. (1963). The underlying geostrophic currents that move eastward across the Pacific between 40° and 50°N, divide near 135°W, with part circling anticlockwise around the Gulf of Alaska and then dispersing into the Bering Sea, and part turning south to the California coast (Tully and Barber, 1960). A cold core with upward entrainment of deep water occurs near the dynamic centre of the Alaskan Gyre, at about 53°N,150° to 160°W, (Uda, 1963; Reid, 1962; Tabata, 1965). Off the Canadian coast during August 1955, Bennett (1959) found indications of a southeast flow when the reference level was 200 db, but a northwest flow when referred to 2000 db.

It is suggested that a major cause of zooplankton variation downstream of the division point is the change in proportion of surface-layer, wind-driven water (Ekman transport) that is swept southward out of the subarctic circulation. Drift bottles have crossed the underlying geostrophic flow (Dodimead and Hollister, 1958). (The total transport may also vary in the same sense in most years, which would reinforce the total effect.) As may be seen in Fig. 1, the zooplankton distributions in the eastern North Pacific Ocean given by Reid (1962) and by Parsons et al. (1966) indicate that zooplankton are concentrated around the gyre and down the west coast of the U.S.A. Reid's explanation for the variation of zooplankton in the California Current as being related to the varying influence of subarctic water appears correct. The evidence for this mechanism fits the suggested time sequence when the rate of geostrophic flow of 4 miles per day for 300 days between 50°N,140°W and lower California is taken into account. In the same way that Namias found air masses being advected 1400 miles from 50°N,150°W in a few days, water masses may be advected 1400 miles in 1 year. The converse flow around the gyre (Table II) agrees with the assessment of variation in circulation from 1955 to 1959 by Dodimead et al. (1963). In 1955 the westward extension of the Alaskan Stream was least. In 1958, there was an apparent increase in the amount of water flowing into the Alaskan Stream, and a westward extension of comparatively warm water, with slightly lower salinity, in the central Bering Sea. Again, the proportion of surface water carried along on the total flow should be considered to be a major cause of annual variation in the animal population downstream.

The location, 50°N,140°W, is between the dynamic centre of the Alaskan Gyre, the encircling zooplankton concentration and the area of division of the eastward moving ocean water (Fig. 1). The southerly components of surface flow can be related to nutrient supply from the northwest and the easterly components to zooplankton removal to the southeast. Favorite (1964) clearly showed that easterly components at 50°N,160°W can be related directly to the eastward transport and inversely to salinity along 50°N.

A change of emphasis on known mechanisms of environmental effect on biological productivity is advanced here. Rather than concentrate on the effects of divergence and upwelling, and the introduction of new water to the surface in the area of zooplankton collection, consider those water masses which are swept away in the divergent process. These nutrient-rich waters maintain their identity as "biologically conditioned" volumes capable of transmitting biological as well as physical and chemical events to other areas.

DISCUSSION

The annual variations in zooplankton abundance at Station P and in the western Bering Sea appear to be due to a relatively simple mechanism. The coastal California case appears more complex and warrants a more detailed discussion.

Dodimead et al. (1963) concluded that "the main surface flow out of the subarctic, which is, for the most part, *closed*, occurs at the subarctic-subtropic boundary through the California current system." *This paper* takes for granted that zooplankton and nutrients around the Alaskan Gyre will be carried with the surface waters. Furthermore, it would appear to follow from the above quotation that calculated surface transport at a point in the vicinity of the division between Gyre flow and the upstream end of the California Current would be related to the annual variations in surface water escaping out of the subarctic circulation.

It may be argued that weather and oceanographic systems are linked together so that the appearance of an "upstream" influence is an artifact due to the persistence, for 12 months in the areas of zooplankton collections, of the effects of local upwelling and insolation. Namias (1965a, b) found that the period of the persistence of wind-induced temperature effects in the ocean are of the order of a month. Weather conditions off California do vary with Gulf of Alaska barometric pressure (from which Fofonoff Ekman transport for that area was calculated) with short-period lags of the order of a week (Namias, 1953). Water masses off California would have to be conserved for 12 months to exhibit the 12-month lag found between the weather (wind) and the zooplankton if the effect is considered to be due to static local conditions. Because the annual variations in zooplankton off Southern California are due to variations in species additional to the subarctic ones, then transport mechanisms that will allow for recirculation of southern species into the offshore California Current, and also allow for annual variation in their abundance, need to be examined.

There is continuity between the California Current and the Equatorial Currents (Reid et al., 1958). If water and its associated nutrients and biota are conserved for 12 months along the North American shore, then flow patterns other than the simple offshore transport usually diagrammed for coastal upwelling must be present. Reid et al. (1958) and Reid (1960) showed that upwelling is centered on Cape Mendocino. A narrow current flows northward at half a knot, no more than 40 miles offshore, just below the surface or at the surface when northerly winds are absent. This current carried Equatorial Pacific water northward. Doe (1955) gave the directions of flow as principally northwestward along the British Columbia coast with upwelling taking place under the influence of northwest winds in the same period. He found southern water present in this flow. Budinger and Enbysk (1960) confirm the northward transport of Equatorial Pacific water, for they found it comprised 10 to 20% of the water at the Cobb Seamount in 46°40′N, 130°50′W. This position is in the southward surface circulation relative to 1000 db (Dodimead et al., 1963).

It is suggested that some upwelled water along the coast is first carried north, is entrained westward into the California Current, and that the mean transport of entrained coastal water north and south takes 1 year. The offshore abundance of northern species of zooplankton between 40°N and 49°N at longitude 128°W (Reid, 1962) shown in Fig. 1 could be related to this mechanism. Annual variations in summer upwelling could be related directly to surface flow out of the subarctic through the linked weather systems reported by Namias (1953) and discussed by Reid (1960).

The following quotations from Reid et al. (1958) support the foregoing: "The California Current system contains a band of cold water reaching from high latitudes far down the coast of North America. It brings in waters of relatively high phosphate content, in sharp contrast to the waters farther offshore. Along the coast this higher value of phosphate is reinforced by the upwelling of deeper waters whose nutrient properties have not been exhausted. From the south, at a depth of about 200 metres, another current moves northward carrying nutrient material which is transported up the coast immediately below the surface layer and becomes available to the plants by mixing and upwelling. The Equatorial Pacific water can still be identified in a state of transition along the coast, possibly as far north as the Gulf of Alaska and in the offshore waters as far as 40°N latitude."

Balech (1960) concluded that the distribution of phytoplankton off southern California in the spring of 1958 indicated a transport of populations from a centre of subtropical or even tropical water, and a cyclonic circulation adjacent to the California coast.

Berner (1960) reported on the 1957 and 1958 distribution of northern and southern forms of tunicates in southern coastal California waters. Brinton (1960) reported on comparable distributions of euphausiids. In 1958 the southern forms were distributed farther north than in 1957; in 1957 the northern forms were farther south than in 1958. The densities of the combined species (Table I) were greater in 1957 than in 1958. Densities were low in both 1958 and 1959.

Apparently advection of southern water does not lead to an increase in density of zooplankton, either in the same or in the following year. The evidence reinforces the view that southern forms are always recruited to the California sampling area, but that densities of all species are related to the advection of northern water.

NUMERICAL VALUES

Although Fofonoff and Dobson (MS, 1963) warn that numerical equivalence of computed and observed transports is not anticipated, the values used are not unreasonable. From Fig. 3 it can be seen that there are pulses in the southerly transport at the upstream end of the California Current. These are not cyclic but vary from 3 to 5 years apart. The mean initial direction of the resultant vector of zonal and meridional Ekman transport is 147°. This is 37° to the right of the storm track reported by Namias (1953). The monthly mean Ekman transport for January to August is 368 metric T/km per second. The southerly component averaged 330 metric T/km per second. The average current speed calculated for the top 100 m of the ocean is 0.15 miles/day. This speed, if correct, is small. It was noted earlier that southerly drift varied consistently over some 20 degrees of longitude (140° to 160°W), or 900 miles at latitude 50°N. During the zonal geostrophic transport in this area, large volumes of water could be shifted *en masse* along a great portion of this particular latitude. The distance for surface water to be shifted across the geostrophic flow and out of the subarctic region is feasible, if all Ekman transport were considered to be concentrated in the upper 10 m. The argument for such surface transport would be assisted by the fact that in summer, there is a nearly isothermal layer corresponding to the isohaline layer, extending to about 30 m depth (Dodimead et al., 1963). The mean speed of the currents during the period considered would then be 1.5 miles per day. In years of large transport the speed would be 3 miles per day.

CONCLUSION

The mode of nutrient and zooplankton dispersal suggested here is a simple one. Beklemishev's (1961) description indicates some of the real complexity. He stated: "There is a continuous carrying away by currents in secondary water masses. Communities are being constantly formed in some regions and disintegrating in others." Nevertheless, in recent years some 50 to 70% of the variance (equal to the square of the correlation coefficients) of zooplankton collected in three areas of the North Pacific is accounted for by a single factor, that is, surface transport in the Gulf of Alaska.

ACKNOWLEDGMENTS

The author thanks Drs J. P. Tully and M. Waldichuk for their support during this study and his colleagues at the Pacific Oceanographic Group, particularly A. J. Dodimead and C. D. McAllister, for their helpful discussions and criticisms. Mr Jerome Namias and Mr R. H. Gelhard of the Extended Forecast Division of the U.S. Weather Bureau have been kind enough to supply copies of sea level pressure cards. Mr A. G. Fowler and Dr C. Froese of the Computing Centre at the University of British Columbia made the detailed adjustments required for modified input and output formats of the transports computed.

REFERENCES

Balech, Enrique. 1960. The changes in the phytoplankton population off the California coast. Calif. Coop. Ocean. Fish. Invest. Rept., **7**: 127–132.

Beklemishev, K. V. 1961. Spatial structure of plankton communities in relation to type of ocean currents. Range boundaries of pelagic plankton animals in the northern part of the Pacific Ocean. Okeanologiya, **1**(6): 1059–1072. Biol. Abstr., **41**(6) Pt. 1: 21428.

Bennett, E. B. 1959. Some oceanographic features of the northeast Pacific Ocean during August 1955. J. Fish. Res. Bd. Canada, **16**(5): 565–633.

Berner, Leo D. 1960. Unusual features in the distribution of pelagic tunicates in 1957 and 1958. Calif. Coop. Ocean. Fish. Invest. Rept., **7**: 133–135.

Brinton, Edward. 1960. Changes in the distribution of euphausiid crustaceans in the region of the California Current. Ibid., **7**: 137–146

Budinger, T. F., and Betty J. Enbysk. 1960. Cobb Seamount, a deep-sea feature off the Washington coast. Dept. Oceanog., Univ. Wash. Tech. Rept. No. 60. 88 p.

Corlett, John. 1964. Winds, currents, plankton and the year-class strength of cod in the western Barents Sea. ICNAF Environ. Symp. Rome, January 1964. Contrib. B-10. Serial No. 1212. 10 p.

Dodimead, A. J., F. Favorite, and T. Hirano. 1963. Review of oceanography of the subarctic Pacific region. Bull. Intern. North Pacific Fish. Comm., No. 13. 195 p.

Dodimead, A. J., and H. J. Hollister. 1958. Progress report of drift bottle releases in the northeast Pacific Ocean. J. Fish. Res. Bd. Canada, **15**(5): 851–865.

DOE, L. A. E. 1955. Offshore waters of the Canadian Pacific Coast. Ibid., **12**(1): 1–34.

EBER, LAURENCE E., AND OSCAR E. SETTE. 1959. Indices of mean monthly geostrophic wind over the North Pacific Ocean. U.S. Fish Wildlife Serv., Spec. Sci. Rept. Fish. No. 323. 108 p.

FAVORITE, F. 1964. Research by the United States. Oceanography. Intern. North Pacific Fish. Comm., Ann. Rept. 1963. p. 141–148.
1965. Drift bottle experiment in the northern North Pacific Ocean. 1962–1964. J. Oceanogr. Soc. Japan (1964), **20**(4): 160-167.

FAVORITE, F., D. FISK, AND W. J. INGRAHAM, JR. 1965. First transponding oceanographic buoys in the Pacific. J. Fish. Res. Bd. Canada, **22**(3): 689–694.

FOFONOFF, N. P. 1962. Machine computations of mass transport in the North Pacific Ocean. Ibid., **19**(6): 1121–1141.
MS, 1960. Transport computation for the North Pacific Ocean 1955–60. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanog. Limnol.). No. 77–80.

FOFONOFF, N. P., AND F. W. DOBSON. MS, 1963. Transport computations for the North Pacific Ocean. 1950–1954, 1962. Ibid., No. 149–153. 164 p.

FROESE, C. 1963. Computation of mass transport in the ocean from atmospheric pressure data FORTRAN I Program for IBM 1620 computer. Ibid., No. 163. 16 p.

FISHERIES AGENCY OF JAPAN. 1964. Report on research by Japan for the International North Pacific Fisheries Commission during the year 1963. Intern. North Pacific Fish. Comm., Ann. Rept. 1963. p. 60–112.

LEBRASSEUR, R. J. MS, 1965. Seasonal and annual variations of net zooplankton at Ocean Station P 1956–1964. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanogr. Limnol.) No. 202. 162 p.

MCALLISTER, C. D. 1961. Zooplankton studies at Ocean Weather Station " P " in the northeast Pacific Ocean. J. Fish. Res. Bd. Canada, **18**(1): 1–29.

MCGOWAN, J. A. 1960. The relationship of the distribution of the planktonic worm, *Poeolorus meseres* Heath, to the water masses of the North Pacific. Deep-Sea Res., **6**(2): 125–139.

NAMIAS, JEROME. 1953. Thirty-day forecasting: a review of a ten-year experiment. Meteorol. Monographs, **2**(6): 83 p.
1965a. Short-period climatic fluctuations. Science, **147**(3659): 696–706.
1965b. Macroscopic association between mean monthly sea-surface temperature and the overlying winds. J. Geophys. Res., **70**(10): 2307–2318.

PARSONS, T. R., L. F. GIOVANDO, AND R. J. LEBRASSEUR. 1966. The advent of the spring bloom in the eastern subarctic Pacific Ocean. J. Fish. Res. Bd. Canada, **23**(4): 539–546.

REID, JOSEPH L., JR. 1960. Oceanography of the northeastern Pacific Ocean during the last ten years. Calif. Coop. Ocean. Fish. Invest. Rept., **7**: 77–90.
1962. On circulation, phosphate-phosphorus content and zooplankton volumes in the upper part of the Pacific Ocean. Limnol. Oceanogr., **7**(3): 287–306.

REID, J. L., JR., G. I. RODEN, AND J. G. WYLLIE. 1958. Studies of the California Current system. Calif. Coop. Ocean. Fish. Invest. Progr. Rept. 1 July 1956 to 1 Jan 1958. p. 27–56.

ROBERTSON, D. G., J. WONG, A. R. STANLEY-JONES, AND H. WILKE. 1965. Oceanographic atlas of Ocean Weather Station " Papa " 1956–1963. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanogr. Limnol.). No. 187.

SCHAEFER, MILNER B. 1961. Tuna oceanography program in the tropical central and eastern Pacific. Calif. Coop. Ocean. Fish. Invest., **8**: 41–44.

SCHELTEMA, RUDOLF S. 1966. Evidence for trans-Atlantic transport of gastropod larvae belonging to the genus Cymatium. Deep-Sea Res., **13**(1): 83–96.

SETTE, O. E. 1957. Nourishment of central Pacific stocks of tuna by the equatorial circulation system. Proc. 8th Pacific Sci. Congr. (1953), **3**: 131–148.

SETTE, O. E., AND J. D. ISAACS. 1960. Editors' summary — Symp. " The changing Pacific Ocean in 1957 and 1958." Calif. Coop. Ocean. Fish. Invest. Rept., **7**: 217.

SVERDRUP, H. U., MARTIN W. JOHNSON, AND RICHARD H. FLEMING. 1942. The Oceans. Prentice-Hall, Inc. x + 1087 p.

TABATA, S. 1965. Variability of oceanographic conditions at Ocean Station " P " in the Northeast Pacific Ocean. Trans. Roy. Soc. Canada, **III** (Ser. IV): 367–418.

THRAILKILL, J. R. 1963. Zooplankton volumes off the Pacific Coast, 1959. U.S. Fish. Wildlife Serv. Spec. Sci. Rept. Fish. No. 414. 77 p.

TULLY, J. P., AND F. G. BARBER. 1960. An estuarine analogy in the sub-Arctic Pacific Ocean. J. Fish. Res. Bd. Canada, **17**(1): 91–112.

UDA, M. 1963. Oceanography of the subarctic Pacific Ocean. Ibid., **20**(1): 119–179.

WICKETT, W. PERCY. MS, 1966. Fofonoff transport computations for the North Pacific Ocean 1962–1965. Fish. Res. Bd. Canada, MS Rept. Ser. (Oceanogr. Limnol.). No. 218–221.

WILLIAMSON, M. N. 1961. An ecological survey of a Scottish herring fishery. Part IV. Changes in the plankton during the period 1949 to 1959. *Appendix* A method for studying the relation of plankton to hydrography. Bull. Marine Ecol., **5**(48): 207–229.

Rapid Germination of *Clostridium botulinum* Type E Spores[1]

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ABSTRACT

Spores of *Clostridium botulinum* type E germinated rapidly under suitably defined conditions of *p*H, temperature, and nutrient concentration. The influence of these factors on the rate of germination is presented graphically on the basis of germination proceeding according to a first-order kinetic equation. The effect of agents associated with food preservation on the germination rate demonstrates the value of a rapid germination technique for screening inhibitors of spore germination and outgrowth. The kinetic data are discussed on the basis of the current knowledge of spore activation.

INTRODUCTION

The establishment of conditions permitting rapid and predictable germination of bacterial spores is desirable from both applied and fundamental standpoints. It is then possible not only to compare the effects of environmental factors and the addition of inhibitory or stimulatory agents on the germination process, but also to arrive at a better understanding of the mechanisms involved in proceeding from a dormant spore to a new and reproducing vegetative cell.

Recently, a number of reports have appeared on the kinetics of spore germination and have demonstrated the usefulness of these studies for the quantitative evaluation of factors influencing spore germination (Hitzman et al., 1957; Treadwell et al., 1958; Woese and Morowitz, 1958; Gibbs, 1964; McCormick, 1965).

For obvious reasons, most reports of kinetic studies have dealt with spores of aerobic bacteria. The following investigation was designed to extend these studies to anaerobes and in particular to spores of *Clostridium botulinum* type E.

Frequently, terms used to designate a specific biological process become inadequate in the light of more recent studies. Germination as it pertains to bacterial spores is one such term. In the terminology of Murrell (1961) three separate stages are evident in the germination process: (i) activation, in which the dormant state is conditioned to allow rapid germination; (ii) initiation, or those processes bringing about the degradation of the spore wall; and (iii) outgrowth to the new vegetative cell. In this report, germination is defined as the breaking of the dormant state with subsequent loss of heat resistance, increase in stainability, decrease in optical density, and phase-contrast darkening (activation + initiation).

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Germination rate measurements made by following the drop in optical density of the germinating culture are in good agreement with loss of heat resistance, degradation of spore coats, etc. (Woese and Morowitz, 1958), and provide the most convenient method for studying the process.

Spore suspensions of *C. botulinum* type E, when incubated under proper conditions, undergo a decrease in optical density (after a short initial lag) to about one-half the initial or zero time value. The plotting function has been described by Woese and Morowitz (1958) for spores of *Bacillus subtilis* as follows:

$$\log \frac{\mathrm{OD}-\mathrm{OD}_f}{\mathrm{OD}_i-\mathrm{OD}_f} \quad \text{versus time} \qquad \ldots\ldots (1)$$

where OD is the optical density at time t; OD_i and OD_f are the initial and final optical densities respectively. In Fig. 1–7 of the text

$$y = \frac{\mathrm{OD}-\mathrm{OD}_f}{\mathrm{OD}_i-\mathrm{OD}_f}.$$

The slope of the curve is taken as the germination rate constant, K_o.

METHODS AND MATERIALS

Stock cultures of *C. botulinum* type E, strain Minnesota, were maintained in the PE-2 medium of Follinazzo and Troy (1954). Trypticase-peptone medium (Schmidt et al., 1962) containing 0.4 and 0.1% final concentrations of glucose and cysteine respectively (sterilized by filtration and added before inoculation) was employed for growth and spore production.

Growth and spore formation were followed spectrophotometrically with a Bausch and Lomb Spectronic 20 and microscopically with a Wild M20 microscope equipped with phase attachment and photoautomat MKa5 assembly. The technique of "culture activation" as described by Zoha and Sadoff (1958) and by Tsuji and Perkins (1962) was used to obtain high spore yields and synchronous sporulation. Water-jacketed, double side-arm culture flasks (spinner-flasks) were obtained from Bellco Glass Inc., Vineland, N.J. The side-arms contained serum stoppers permitting insertion of hypodermic needles for gassing and sampling. The flasks are supplied with a suspended magnetic stirring-bar for mixing. An FD Bench-Scale biO-kulture model fermentor (Fermentation Design Inc., Durham, Penn.) with conventional stirring, automatic foam control and equipped with 7.5- and 14-litre vessels, was employed for large scale production of spores. The inoculum was introduced to the bottom of the vessel by exerting a positive pressure on a spinner-flask containing 1 litre of a 6-hr culture. Fermentation vessels (7.5 litre) were sparged with a mixture of 95% nitrogen: 5% CO_2 at a rate of 0.5 litre/min and stirred at a rate of 20 rpm. Temperature was maintained at 30 ± 0.5 C. Addition of antifoam agent was not required provided the stirring rate was maintained below 30 rpm. Gas outlets from all culture vessels led directly to a fume-hood.

Cultures were collected by batch centrifugation or by continuous-flow centrifugation through $\frac{1}{4}$-inch PVC tubing at 5000 rpm using a Model 2303 Helixtractor (International Equipment Co.). The flow-rate through the tubing was set to 20 ml/min. After centrifugation, the coiled tubing was clamped at the inlet and outlet ends, cut, and removed. Maximum recovery was obtained by washing the cell paste through the tubing with dilute buffer. Expressing the paste by means of the roller-assembly supplied gave poor recovery.

The helixtractor is a completely sealed unit and eliminates the danger of toxic aerosols. The entire unit may be autoclaved after use.

Cells were suspended in sterile 0.01 M phosphate buffer, *p*H 7.0, to 20 times growth concentration, stirred gently for 72 hr at 10 C, centrifuged at 2000 *g* for 30 min, and resuspended to 20 times growth. Spores were freed of sporangia by twice subjecting the suspension to sonic oscillation for 3 min followed by three additional washings in dilute buffer. Spore suspensions were stored at 1–2 C in 0.02 M phosphate buffer, *p*H 7.0. Suspensions stored in distilled water began clumping after 3 months storage.

Spore counts were made with a Petroff-Hausser chamber. Germination rates were determined by following the change in OD at 600 mμ in a Spectronic 20. Measurements were made directly in 14 × 170-mm Thunberg tubes. Unless otherwise stated, the basic germination medium contained the following components: trypticase (BBL), 0.6%; peptone (Difco), 1.0%; glucose, 0.02 M; phosphate buffer, 0.04 M; *p*H 7.0. Spore suspension (1 ml) containing 3×10^8 spores/ml was placed in the side-arm of the Thunberg tube and the contents tipped in at zero time after evacuation and temperature equilibration. The final volume was 5.0 ml.

Tylosin lactate was received as a gift from Dr Bernard Malin of Eli Lilly and Company, Indianapolis, Indiana; 2-(2-furyl)-3- (5 nitro-2-furyl) acrylamide was obtained through the courtesy of Dr Toshiharu Kawabata, National Institute of Health, Shinagawa-ku, Tokyo, Japan.

RESULTS

GERMINATION MEDIUM

Tryptic digests of casein, supplemented with peptones and glucose, are routinely employed for growth and spore production of *C. botulinum*. The assay system, as described in Methods, allows a rapid and complete germination of spores without prior heat-shock treatment. Under optimum conditions, the process is complete in about 1 hr at which time outgrowth and subsequent increase in OD occur. A decreasing OD due to germination concurrent with an increasing OD due to outgrowth introduces some error when establishing rates during the latter stages of incubation; for this reason comparison of initial rates is employed when possible.

Maximum final rates occurred at trypticase concentrations greater than 0.5% (Fig. 1). Initial rates were proportional to the trypticase concentration between 0.5 and 2% and the time required to achieve 50% germination was halved as the concentration doubled.

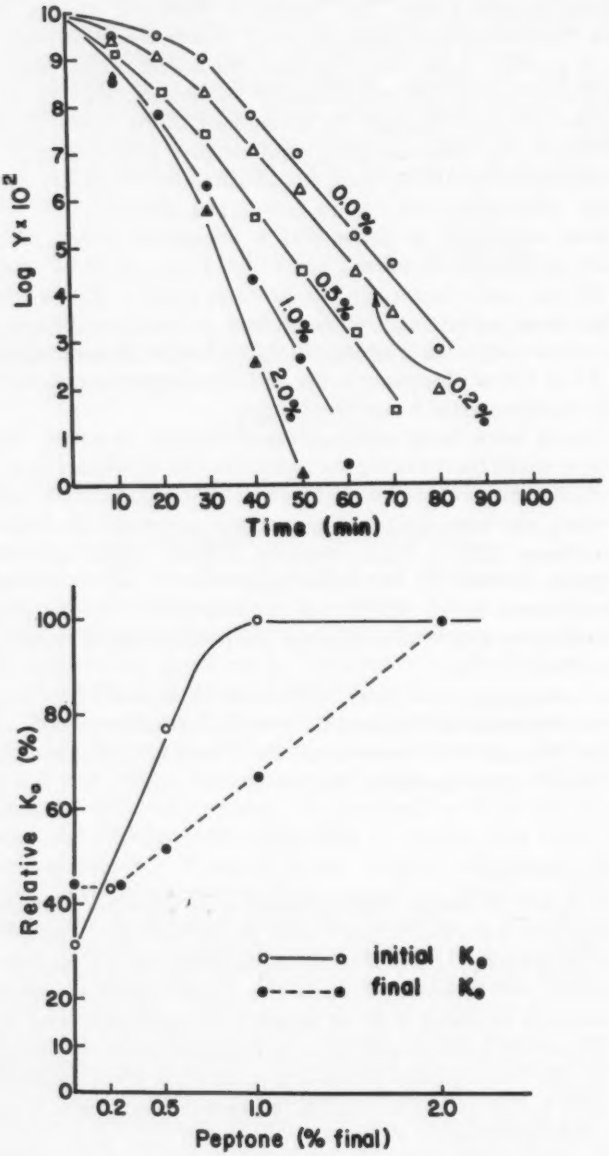

FIG. 1. The effect of trypticase concentrations on spore germination.

The active component(s) in trypticase were not identified but could be partially purified by fractionating the trypticase on Sephadex G-10. The active fraction was of low molecular weight, dialyzable, and heat stable. Amino acid analysis of this fraction revealed the presence of all the common amino acids. Replacing the trypticase of the germination medium with these amino acids in their original concentrations did not allow germination. Interestingly, the fractionated trypticase allowed good outgrowth of spores but sporulation of vegetative cells did not occur, a higher molecular weight fraction of the trypticase being required.

Varying the peptone concentration of the system affected both initial and final rates of germination (Fig. 2), although fairly rapid rates were obtained

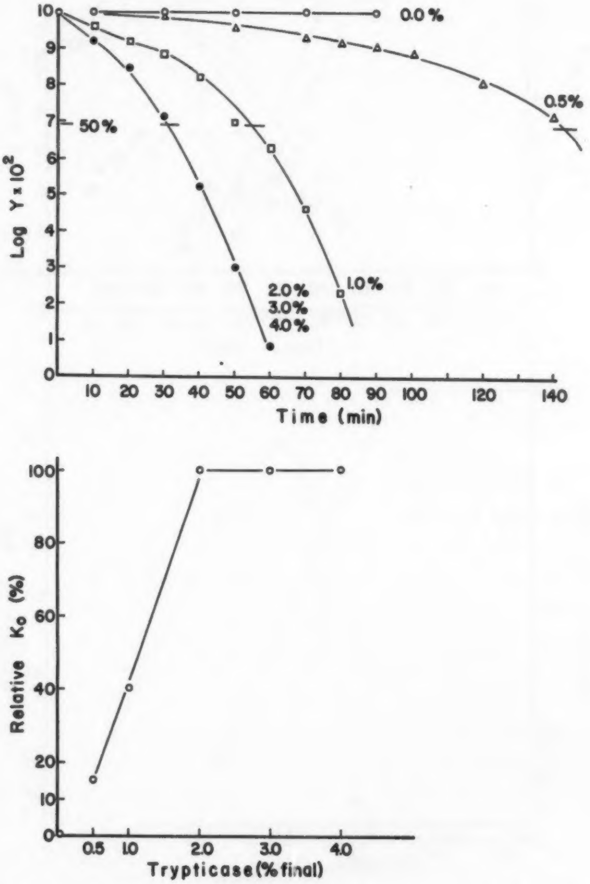

FIG. 2. The effect of peptone concentrations on spore germination.

when peptone was omitted. Maximum initial and final rates occurred at concentrations of 1.0 and 2.0% respectively.

Initial germination rates were not markedly affected by varying glucose concentration (Fig. 3) and proceeded fairly rapidly for a period of 10–20 min in its absence. Final rates, however, were dependent on glucose concentration,

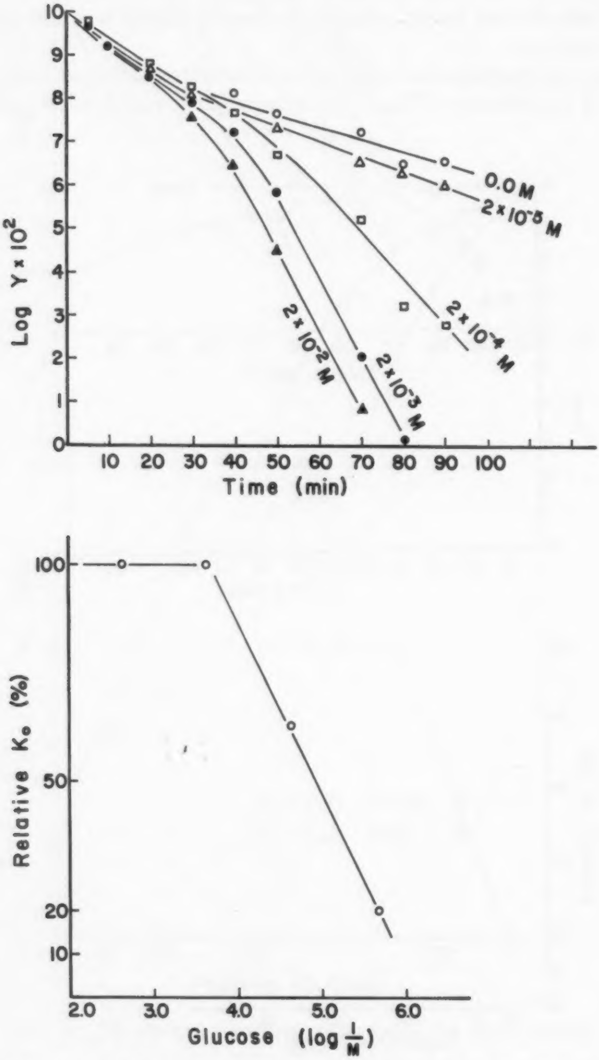

FIG. 3. The effect of glucose concentrations on spore germination.

approaching a maximum at 2 × 10^{-3} M glucose. No glucose could be detected in the basal assay system by means of the glucostat reagent. Outgrowth and spore production proceeded in the absence of either glucose or peptone, although total growth was lower without glucose.

TEMPERATURE

The separation of initial and final rates was also evident on the basis of temperature studies (Fig. 4). The optimum temperature for maximum germi-

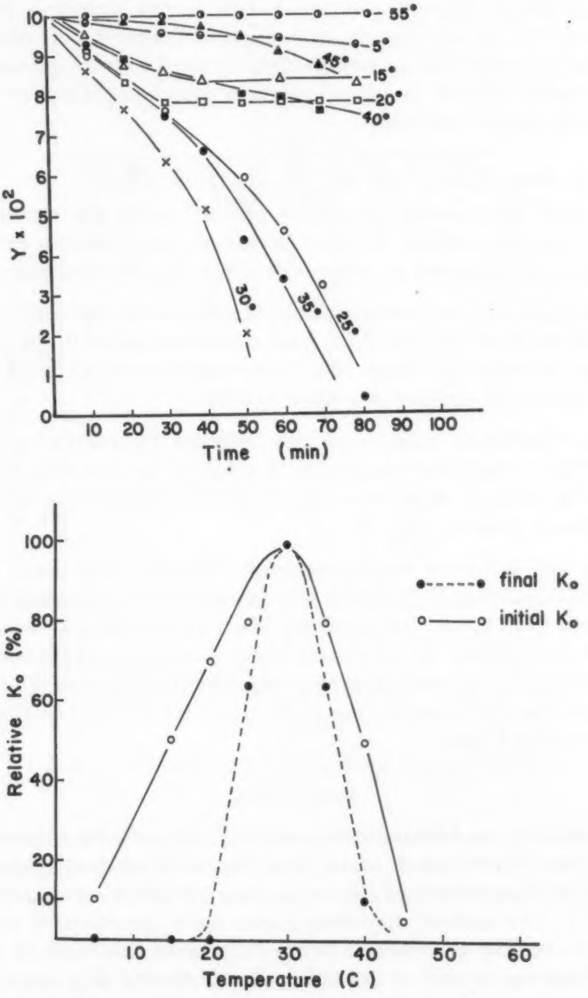

FIG. 4. The effect of temperature on the rate of spore germination.

nation rates was 30 C and slight deviation either above or below 30 C produced a marked reduction in rate. Final rates were considerably more temperature-dependent than initial rates; at 20 C the initial rate was reduced only 30%, whereas the final rate was too low to be measured by this method. Again, at 40 C the initial rate was reduced 50%; the final rate by 90%. The temperature coefficient (Q_{10}) of the germination process (initial rates) was calculated to be 1.4 over the range 5–30 C.

*p*H

The two-phase nature of the germination process was again evident under conditions of varying *p*H (Fig. 5). Maximum initial rates were obtained over a very narrow *p*H range and no germination occurred over an extended period of incubation below *p*H 5.0. If the *p*H of these tubes was raised to 7, rapid and complete germination followed.

EFFECTS OF SOME CHEMICALS ON GERMINATION RATES

The rapid germination procedure proved useful for screening various compounds for their ability to affect the germination process. Frequently the stage of spore development at which this effect was directed was also evident.

Sodium chloride. Low concentrations of sodium chloride slightly stimulated the germination rate (Fig. 6). At a final concentration of 0.1 M (0.58%) salt, the rate was reduced by about 50%; germination was inhibited at 0.5 M or about 3% final concentration of sodium choride.

Sucrose. Maximum rates were not affected by sucrose concentrations to 0.25 M (9%). A final concentration of 1 M (36%) sucrose reduced the germination rate by 50%. A slight reduction in initial rates with increasing sucrose concentrations is evident (Fig. 7).

Tylosin and nitrofuryl furyl acrylamide (NFFA). The assay system was similar to that described in Methods and contained concentrations of the above two inhibitors from 0.1 to 100 ppm. No effect on germination was evident on the basis of rate studies. At all concentrations tested, germination rates were identical to those for control tubes. Tylosin inhibited outgrowth of spores at a final concentration of 1 ppm but not at 0.1 ppm; NFFA inhibited outgrowth at 10 ppm but not at 1 ppm.

DISCUSSION

Under suitable conditions of temperature, *p*H, and nutrient concentration, spores of *C. botulinum* type E undergo rapid germination. After an initial lag the fraction of ungerminated spores was shown to decrease according to first order kinetics. The method described allows for a quantitative evaluation of those factors effecting germination rates. At present this method is used as a basis for comparing natural or defined media for their ability to support spore germination.

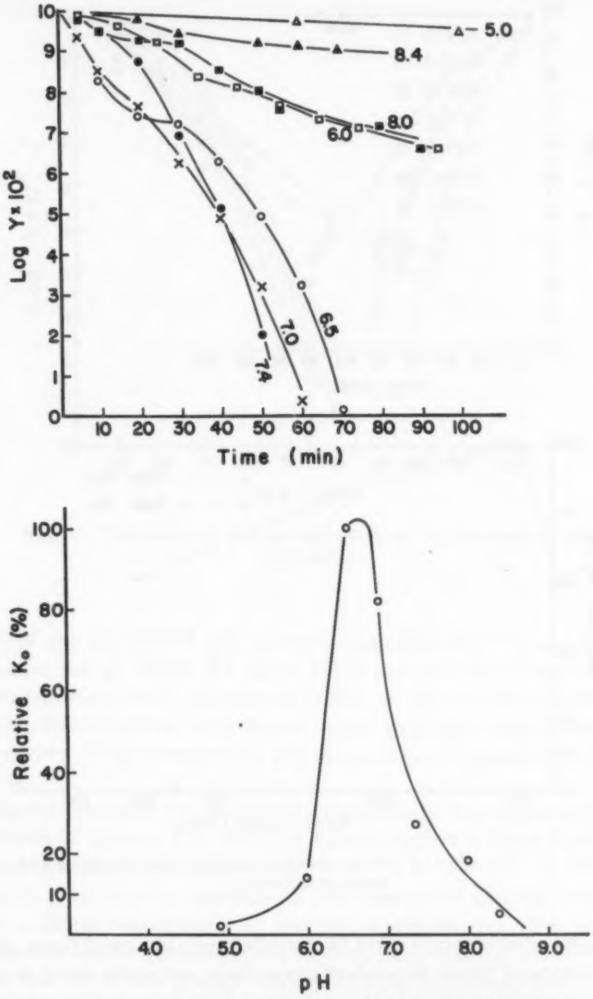

FIG. 5. The effect of *p*H on the rate of spore germination.

An understanding of the kinetics of the germination process, in relation to the curves presented, is complicated by the complexity of the medium employed, and I am currently attempting to obtain similar rapid rates in defined media.

The two-phase nature of the rate curves is especially evident when the *p*H, temperature, or glucose concentration is varied. One would expect that various degrees of dormancy would exist in a given spore suspension, i.e. spores

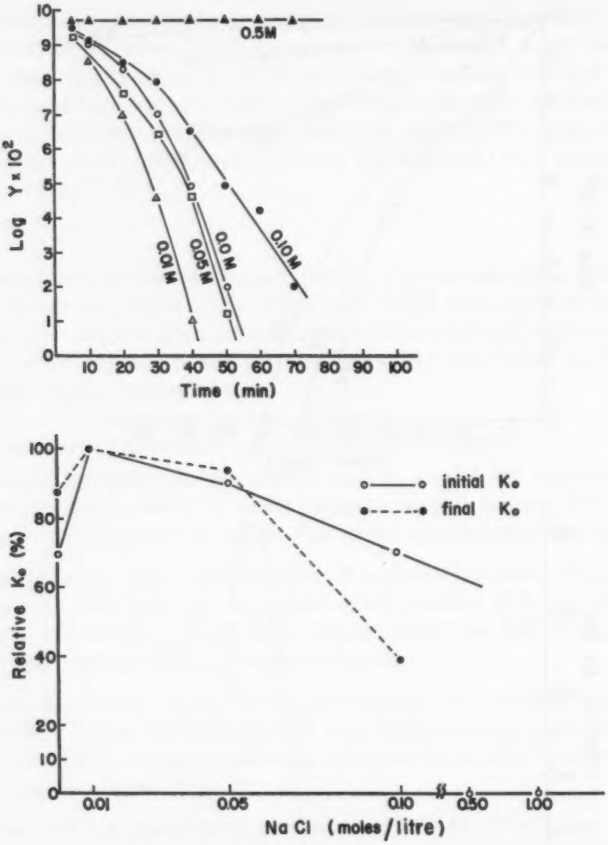

FIG. 6. The effect of sodium chloride concentration on the rate of spore germination.

at various levels of activation, and that under suitable conditions, they would begin to germinate at times dependent upon their particular level of activation. It might also be anticipated that the minimum level for subsequent germination would be temperature-dependent and that at 20 C, for example, only those spores which were at a sufficient level of activation for that temperature would enter the initiation step. The initial slower rates observed could then be the result of a progressive germination of spores beginning with the highly activated spores and proceeding down to the less activated levels. Similar relationships could explain the observed *p*H effect, where dissociation of various active groups in or on the spore coat would be proportional to the degree of activation. In this respect I have observed that freshly prepared spores display longer lag periods before obtaining maximum germination rates than do aged preparations.

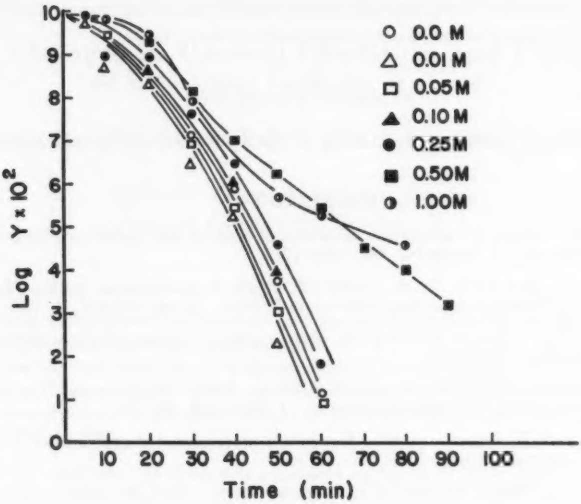

FIG. 7. The effect of sucrose concentration on the rate of spore germination.

The length of the lag period was directly proportional to storage time at 4 C to a minimum lag at about 10 days. High sucrose concentrations would be expected to interfere with the permeability of the activated spore (Lee and Ordal, 1963). The observed lowering of initial and final rates by sucrose would support the view of the presence in the test system of spores at different levels of activation.

Trypticase contains the essential components for complete germination and outgrowth of spores. The effect of concentration is most readily observed with respect to initial germination rates.

Although germination proceeds in the absence of glucose, maximum final rates show a direct relationship to glucose concentration. One possible explanation, presently under consideration, might be that during the activation step a substrate is formed which is itself utilized during initiation; the rate of formation of the substrate might then determine the overall germination rate.

The use of tylosin lactate in food processing for the prevention of growth and toxin production of *C. botulinum* type E has been discussed by Sheneman (1965). Nitrofuryl furyl acrylamide is presently used as a preservative of fish paste products in Japan (Okitsu et al., 1964). Both agents inhibited spore outgrowth without affecting the germination rate. On the basis of glucose oxidation and concurrent methylene blue reduction, catabolic activity of the germinated spore does not appear to be impaired. This is in accordance with previous reports on the action of agents interfering with protein synthesis, nucleic acid synthesis, or membrane transport on germinated spores (Costilow, 1962). Both compounds examined here should prove useful for studies associated with germination where separation from outgrowth is required.

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REFERENCES

Costilow, R. N. 1962. Fermentation activities of control and radiation-killed spores of *Clostridium botulinum.* J. Bacteriol., **84**: 1268–1273.

Follinazzo, J. F., and V. S. Troy. 1954. A simple bacteriological medium for the growth and isolation of spoilage organisms from canned foods. Food Technol., **8**: 280–281.

Gibbs, P. A. 1964. Factors affecting the germination of spores of *Clostridium bifermentans.* J. Gen. Microbiol., **37**: 41–48.

Hitzman, D. D., H. O. Halvorson, and T. Ukita. 1957. Requirements for production and germination of spores of anaerobic bacteria. J. Bacteriol., **74**: 1–7.

Lee, W. H., and Z. J. Ordal. 1963. Reversible activation for germination and subsequent changes in bacterial spores. Ibid., **85**: 207–217.

McCormick, N. G. 1965. Kinetics of spore germination. Ibid., **89**: 1180–1185.

Murrell, W. G 1961. Spore formation and germination as a microbial reaction to the environment. Symp. Soc. Gen. Microbiol., **11**: 100–150.

Okitsu, T., T. Kawabata, and T. Kozima. 1964. Features of the causative organisms of deterioration of fish sausage. 1. Effect of some food preservatives on the growth of causative bacteria. Bull. Japan. Soc. Sci. Fish., **30**: 63–68.

Schmidt, C. F., W. K. Nank, and R. V. Lechowich. 1962. Radiation sterilization of food. II. Some aspects of the growth, sporulation, and radiation resistance of spores of *Clostridium botulinum* Type E. J. Food. Sci., **27**: 77–84.

Sheneman, J. M. 1965. Prevention of Type E *Clostridium botulinum* toxin formation in smoked whitefish chubs with tylosin lactate. Ibid., **30**: 337–343.

Treadwell, P. E., G. J. Jann, and A. J. Salle. 1958. Studies on factors affecting the rapid germination of spores of *Clostridium botulinum.* J. Bacteriol., **76**: 549–556.

Tsuji, K., and W. E. Perkins. 1962. Sporulation of *C. botulinum.* I. Selection of an aparticulate sporulation medium. Ibid., **84**: 81–85.

Woese, C., and H. J. Morowitz. 1958. Kinetics of the release of dipicolinic acid from spores of *Bacillus subtilis.* Ibid., **76**: 81–83.

Zoha, S. M. S., and H. L. Sadoff, 1958. Production of pure spores by a putrefactive anaerobe. Ibid., **76**: 203–205.

Seasonal Changes in General Condition and Lipid Content of Cod from Inshore Waters[1,2]

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ABSTRACT

The seasonal variations in lipid content and general "condition" of cod, *Gadus morhua* L., from an inshore population have been determined.

Four female and four male fish were chosen from 20 live cod brought in monthly from Terence Bay, Halifax County, Nova Scotia, the fish were pooled and the lipid content determined on the flesh, livers, and gonads. The flesh lipids varied from 0.57% to 0.74% and the oil content of the livers from 15% to 75%.

The general "condition" of the fish as expressed by $K_F = \frac{\text{weight} \times 100}{(\text{length})^3}$, the liver condition by $K_L = \frac{\text{weight of liver}}{\text{weight of fish}} \times 100$, and the fat content of the liver all showed seasonal variation with maxima in the fall and minima in the spring. K_F varied from 0.79 to 1.05 and K_L from 1.0 to 4.5. No seasonal variation in the amount of flesh lipids could be detected.

INTRODUCTION

SEVERAL AUTHORS have studied the seasonal changes occurring in lipids, proteins, and water in cod from various areas. In initiating the studies in this report, believed to be on an isolated population, we were chiefly interested in the fatty acid compositions of flesh and organs which have not previously been extensively studied and which are published in the next paper of this series (Jangaard et al., 1967).

Graham (1923) published some excellent work on the seasonal variation in conditions ($K_F = \frac{\text{weight} \times 100}{(\text{length})^3}$) with sexual maturity in the North Sea cod. He found that individual condition varied widely in large samples of fish, but in general increased from June until October, then decreased until spawning was completed in March–May. He also found that the cyclical changes in condition were approximately the same for mature as for immature fish. The liver condition ($K_L = \frac{\text{weight of liver} \times 100}{\text{weight of fish}}$) was found to be markedly greater for the mature female fish than for the mature males.

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Bogucki and Trzesinski (1949) studied the variations in water and lipids in Baltic cod and reported that these indices were linked to the annual reproductive cycle. Their method for lipid extraction (Soxhlet) did not give conclusive evidence for seasonal changes in the flesh lipids, but the percentage fat in the liver reached a minimum shortly after spawning. Lovern et al. (1959) could not detect any definite seasonal trend in muscle lipids, whereas Dambergs (1963, 1964) employing an isopropanol extraction method found a definite seasonal variation in the muscle lipids of Nova Scotian cod with a maximum in July–September and a minimum in April. It is possible that Dambergs' method will extract some of the lipoproteins since his values were generally higher than values obtained by Bligh and Dyer's (1959) method. MacCallum et al. (1964) found that the average total lipid was higher in Newfoundland trap cod (0.91 ± 0.014%) caught in the summer than in fish caught in late fall (0.63 ± 0.10%).

The inshore cod from the South Shore of Nova Scotia has been found to spawn from March to May although a fall spawning population in Halifax Harbour has been described (McKenzie, 1940, 1956). McKenzie also found from tagging experiments that little migration occurs between inshore and offshore bank areas and that several populations of inshore cod could be classified by vertebral counts (McKenzie and Smith, 1955). It is, therefore, probable that all the fish sampled in this study were from the same population.

EXPERIMENTAL

The fish were caught by handline and brought alive to this laboratory by truck from Terence Bay, Halifax County, Nova Scotia. The sampling procedure as applied to the lipid research program was carried out as follows.

Twenty of the larger fish were chosen, anaesthetized with MS-222, a numbered tag attached, and whole weight and overall length recorded. The fish were then killed, gutted, filleted, and the livers and gonads weighed. Four female and four male fish of uniform size were chosen, whenever possible, for the lipid determinations. In addition, several larger fish were analyzed singly when available.

The fillets and livers respectively from each lot of four fish were pooled and appropriate uniform samples taken for lipid extraction. The livers were ground with anhydrous sodium sulfate and extracted twice with ethyl ether. The fillets and gonads were extracted by the method of Bligh and Dyer (1959).

The flesh lipids were chromatographed on silicic acid, the neutral lipids eluted with chloroform, and the phospholipids with methanol. Unsaponifiable matter and iodine values were determined according to the Official Methods of the American Oil Chemists' Society.

This various lipids were then esterified and the fatty acid composition determined by gas–liquid chromatography as described elsewhere (Jangaard et al., 1967).

RESULTS AND DISCUSSION

SAMPLING

When this study was undertaken, it was hoped that a good supply of large, mature cod would be available each month. However, it soon became evident that this would not be feasible with the type of fishing employed (handlining), and in several of the monthly samples only immature fish were available. In Table I the monthly average sizes and weights of the samples are listed by sex.

As far as possible an attempt was made to select fish of uniform length for each monthly sample. The difficulty in securing larger fish is partly because these fish tend to move to deeper water in winter and are closest to shore in July (McKenzie, 1956). Also the fish are reluctant to take bait or the jig near spawning time. This is clearly shown in the samples obtained by bottom trawling by the m/v *Harengus* in the same areas on Feb. 24 and March 23, 1964 (Table I). These larger fish were ripening or ripe, and the stomach contents indicated that they were feeding on shrimp and fish even though they could not be caught on handlines.

TABLE I. Average lengths and weights of cod sampled for lipid determinations (four female and four male).

	4 female		4 male	
Date collected	Average length (*cm*)	Average weight (*g*)	Average length (*cm*)	Average weight (*g*)
Nov. 6, 1962	44	860	51	1190
Dec. 3	55	1560	49	1070
Jan. 7, 1963	56	1630	53	1430
Feb. 2	54	1330	54	1450
March 5	55	1340	54	1500
April 7	58	1890	62	2300
May 7	54	1480	57	1600
June 6	61	2220	54	1520
July 2	62	2380	57	1960
Aug. 1	64	2830	62	2350
Sept. 5	58	1840	57	1760
Oct. 1	58	2070	60	2130
Nov. 5	66	2950	63	2300
Dec. 5	57	1650	54	1590
Jan. 5, 1964	51	1310	53	1360
Feb. 11	50	1040	53	1300
March 4	63	2060	61	2050
	Fish caught by bottom trawl in same area			
Feb. 24, 1964	94	9500	87	8090
March 23, 1964	69	3660	69	3240

"CONDITION" OF FISH

The seasonal variation in condition or fatness of the fish can be measured by several methods. In addition to liver fat or lipid content, the following formula was employed: $K_F = \frac{\text{weight of fish} \times 100}{(\text{length})^3}$ where weights are given in grams and lengths in cm. A formula $K_L = \frac{\text{weight of liver} \times 100}{\text{weight of fish}}$ was used to calculate liver "condition."

In Fig. 1 these indices are plotted monthly and, as can be expected in such a small sample, considerable variation can be found. In addition to the average for the four female and four male fish, the average for the whole 20 fish sample is plotted.

The maxima were found in late summer and fall, July to November, both in overall condition (K_F), and in liver condition (K_L). The indices then dropped off and were at a low level until spawning was completed in March–May and the fish resumed heavy feeding in June. The horizontal line drawn through the graphs represents the arithmetic mean of all 20 monthly samples.

The importance of sampling fish of uniform size can be seen in Fig. 1

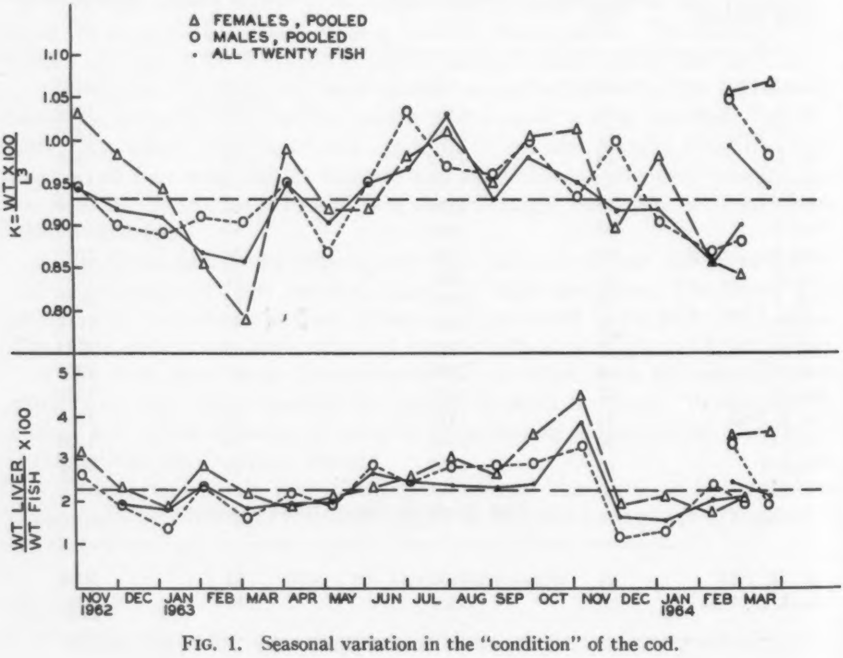

FIG. 1. Seasonal variation in the "condition" of the cod.

in the points marked on Feb. 23 and March 24, 1964. These points represent the large, trawl-caught fish from the same area, and those indices indicate a much better condition than found for the regular monthly samples. The same factors probably influenced the values for November 1963, since the fish sampled in that month were larger than usual (Table I).

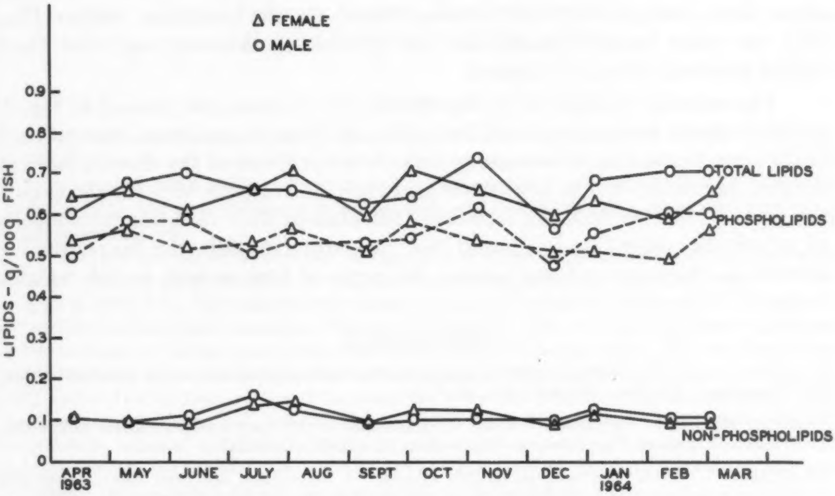

FIG. 2. Lipid content of cod flesh.

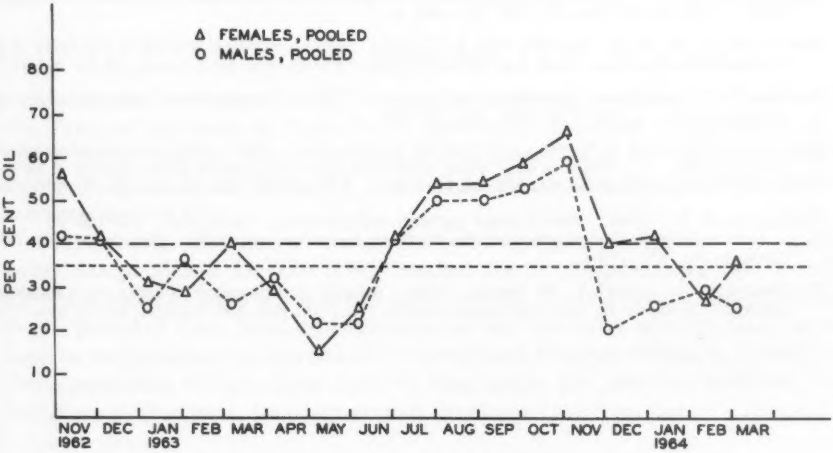

FIG. 3. Percentage oil in cod livers.

Fat or Lipid Content

In Fig. 2 the lipid content of the flesh together with the amount of phospholipids and non-phospholipids (neutral lipids) are plotted. No definite seasonal variation could be established although Dambergs (1964) found that a maximum occurred in July–September and a minimum in March–May in lipids of cod from the same area. The highest values found in our studies were for the female fish caught in October 1963 and for the male fish from November 1963. Although these dates coincide with the maxima found for the condition indices (K_F, K_L), the other monthly results are too variable to definitely say that these values represent seasonal maxima.

The seasonal changes in the fat content of the livers are plotted in Fig. 3. A very definite increase occurred from May to June; a maximum was reached in October–November, then a sharp drop to lower levels as the development of the gonads increased. The arithmetic mean for the year was 40% for the female fish and 35% for the male but individual fish showed wide variation from month to month. In general, it was found that the larger the fish, the higher the fat content of the liver and the greater the ratio of liver weight to fish weight.

REFERENCES

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. Physiol., **37**: 911–917.

BOGUCKI, M., AND P. TRZESINSKI. 1949. Fluctuations in the water and fat content of the cod. J. Conseil, Conseil Perm. Intern. Exploration Mer, **16**(2): 208–210.

DAMBERGS, N. 1963. Extractives of fish muscle. 3. Amounts, sectional distribution and variations of fat, water-solubles, protein and moisture in cod (*Gadus morhua* L.) fillets. J. Fish. Res. Bd. Canada, **20**: 909–918.

1964. Extractives of fish muscle. 4. Seasonal variations of fat, water solubles, protein and water in cod (*Gadus morhua* L.) fillets. Ibid., **21**: 703–709.

GRAHAM, M. 1923. The annual cycle in the life of the mature cod in the North Sea. Min. Agr. Fish. Fish. Invest., Ser. II, Vol. VI. No. 6.

JANGAARD, P. M., R. G. ACKMAN, AND J. C. SIPOS. 1967. Seasonal studies of the fatty acid composition of cod liver, flesh, roe, and milt lipids. J. Fish. Res. Bd. Canada, **24**(3): 613–627.

LOVERN, J. A., JUNE OLLEY, AND HELEN A. WATSON. 1959. Changes in the lipids of cod during storage of cod. J. Sci. Food Agr., **10**: 327–337.

MACCALLUM, W. A., H. S. SHIEH, AND DOROTHY A. CHALKER. 1964. Polyphosphate treatment of frozen cod. 2. Effect of drip, yield, lipid hydrolysis and protein extractability in twice-frozen Newfoundland summer trap and fall cod. J. Fish. Res. Bd. Canada, **21**(3): 539–548.

MCKENZIE, R. A. 1940. Nova Scotian autumn cod spawning. Ibid., **5**(2): 105–120.

1956. Atlantic Cod tagging off the southern Canadian mainland. Bull. Fish. Res. Bd. Canada, No. 105. 93 p.

MCKENZIE, R. A., AND G. F. M. SMITH. 1955. Atlantic cod populations along the Canadian mainland as shown by vertebral count studies. J. Fish. Res. Bd. Canada, **12**(5): 698–705.

Seasonal Changes in Fatty Acid Composition of Cod Liver, Flesh, Roe, and Milt Lipids[1,2]

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ABSTRACT

The fatty acid compositions of lipids from an isolated population of inshore cod (*Gadus morhua* L.) caught near Terence Bay, Nova Scotia, were determined each month on pooled samples of representative fish. Cod liver oil was studied over a period of 17 months, cod flesh for 12 months with the fatty acid compositions of milt, roe, flesh, and liver lipids being determined from some large, individual fish.

The fatty acids of flesh lipids were independent of sex and showed very little, if any, seasonal changes except a slight variation in the long-chain monounsaturated acid 20:1 (max 2.8% vs. avg 1.6% and 1.1%). This was especially evident in large fish where the maximum (3.7%) coincided with the period of best "condition," August to November. There was also a significant difference between large and medium size fish in that in very large fish the content of 22:6 was much lower (26% vs. 33%). Liver oils in female fish contained increasing amounts of 20:1 (4.5 to 14.9%) and 22:1 (1.8 to 12.3%) fatty acids with increased fat content of the liver, maxima occurring in late summer and fall. In the male fish this seasonal trend was not as obvious. No other acids showed any definite seasonal variation. In the fatty acids of roe and milt lipid no definite relationship could be established between ripening and fatty acid composition. The fatty acids of the milt were similar to the roe except for a higher percentage of 18:1 and lower percentage of 16:1. The unsaponifiable matter was higher in milt than in roe lipids.

These results indicate that due to the large variations that can occur in the fatty acid content of lipids from individual fish, a single analysis could give a fatty acid composition quite different from the average of a large number of determinations.

INTRODUCTION

The seasonal variations in lipid content and general "condition" of cod from the Terence Bay area of Nova Scotia have been described (Jangaard et al., 1967). In the present report the fatty acid composition of the flesh, liver, roe, and milt lipids from these fish is given as determined by gas–liquid chromatography (GLC).

Few studies of seasonal changes in fatty acid compositions of cod lipids have been published. DeWitt (1963) studied the fatty acid composition of cod liver oils brought back by trawlers from various North Atlantic fishing areas over a period of time. Most other analyses of cod lipid fatty acids by GLC have been on single fish or commercial oils. Ackman and Burgher (1964a, b, c) studied the composition of flesh, liver, and roe from single fish and also reviewed the literature to that time. Lambertsen and Braekkan (1965) published a thorough

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study of the fatty acids of cod liver oil and compared their detailed values with those obtained by other workers. They calculated an average cod liver oil fatty acid composition from these data. Olley and Duncan (1965) studied the flesh lipid composition of cod, and Gruger et al. (1964) published analyses of cod liver and flesh fatty acid composition. To the best of our knowledge, no analysis of the fatty acid composition of cod milt lipid has been published.

EXPERIMENTAL

Selection of fish for sampling and lipid extractions were carried out as described elsewhere (Jangaard et al., 1967).

The cod liver oils were transesterified employing 0.5% NaOH as the catalyst. The flesh, roe, and milt lipids were saponified, the nonsaponifiable material removed, and the recovered fatty acids esterified with boron trifluoride–methanol reagent. Iodine values were determined by Wijs' method or by semimicrohydrogenation and the unsaponifiable matter by the American Oil Chemists' Society standard method.

Gas–liquid chromatography of the methyl esters was carried out on a Barber-Colman Model 10 equipped with a model 5121 flame ionization detector except for the first five samples of cod flesh lipids which were determined with an argon ionization detector. Two glass columns were used for each analysis (3 mm inside diam, 6 ft in length) packed, respectively, with 11% EGSS-X and 12% EGSS-Y on 100–120 mesh Gas-Chrom P (Applied Science Laboratories, Inc., State College, Pa.). Tentative identifications of the peaks were made by the log-plot and separation-factor procedures (Ackman and Burgher, 1965) and correction factors for quantitative determination were applied (Ackman and Sipos, 1964).

Cod liver oil samples were stored in a coldroom under nitrogen and analyzed together near the conclusion of the study. The flesh lipids were analyzed as rapidly as possible after extraction since the high degree of unsaturation and small samples precluded extended storage due to risk of oxidation.

RESULTS AND DISCUSSION

SEASONAL VARIATION IN LIVER OIL

The fatty acid composition of cod liver oils determined monthly over a period of 17 months is given in Table I for female and in Table II for male fish.

Fatty acids showing the greatest variation were the long-chain monounsaturated 20:1 and 22:1. These are considered to be accessible metabolic reserves and should therefore reach a maximum when the fat content of the livers is high. In general, this correlation is apparent in the female fish from Tables I and II. The fish in best condition, caught November 1963, had the highest content of 20:1 and 22:1 (14.6 and 12.3%), and the leanest fish the lowest; for instance, the female fish caught in May 1963, with less than 20% fat in the liver had only 4.5% 20:1 and 2% 22:1. The two other major monounsaturated acids,

Table I. Component fatty acids of cod liver oils (four female fish pooled monthly; weight %).

Fatty acid	1962		1963												1964			
	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Average
14:0	2.9	2.7	2.7	3.8	3.8	3.6	1.5	3.4	3.3	3.5	3.6	3.4	4.4	3.9	2.7	3.9	2.6	3.27
14:1ω5[a]	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.15
15:0	0.4	0.3	0.5	0.3	0.4	0.5	0.3	0.4	0.3	0.4	0.3	0.3	0.4	0.6	0.4	0.4	0.4	0.39
16:0	13.2	15.4	14.8	12.8	12.2	15.1	13.3	11.7	13.5	12.9	14.9	12.8	12.2	12.2	13.2	12.1	13.3	13.27
16:1ω7[a]	7.4	7.3	8.3	8.7	11.1	6.8	8.4	11.3	10.8	9.1	7.5	10.4	11.2	11.2	9.4	10.9	10.3	9.41
16:2ω4	0.5	0.4	0.2	0.4	0.6	0.5	0.5	0.6	0.5	0.2	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.45
16:3ω4	0.3	0.1	0.1	0.2	0.3	0.2	0.2	0.4	0.1	0.2	0.3	0.2	0.5	0.4	0.1	0.1	0.2	0.23
17:0	0.5	0.6	0.7	0.7	0.8	0.7	0.5	0.6	0.5	0.7	0.8	0.7	0.9	0.7	0.7	0.8	1.0	0.70
17:1ω8[a]	0.5	0.4	0.5	0.4	0.6	0.7	0.7	0.6	0.5	0.3	0.6	0.3	0.1	0.5	0.6	0.5	0.8	0.51
18:0	3.5	3.3	2.8	2.2	1.9	2.7	2.5	2.1	2.6	2.6	3.0	2.2	1.9	2.1	3.1	2.8	3.1	2.61
18:1ω9[a]	22.2	21.4	23.5	21.4	23.8	22.2	31.4	20.5	25.8	21.4	28.2	21.8	17.1	20.9	23.7	20.7	23.5	22.91
18:2ω6	1.1	1.2	2.1	1.3	1.1	1.6	0.8	1.3	1.2	1.3	1.4	1.3	1.5	1.9	1.9	1.8	1.7	1.44
18:3ω3	0.6	0.6	0.7	0.7	0.3	0.8	0.4	0.6	0.4	0.6	0.4	0.5	0.8	0.9	0.8	0.9	0.7	0.63
18:4ω3	1.0	0.8	1.1	1.1	0.7	0.9	0.5	0.9	0.6	1.1	0.9	1.3	1.4	1.4	0.9	1.0	1.2	0.99
19:1ω10[a]	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.3	0.1	0.1	0.2	0.3	0.2	0.2	0.18
20:1ω9[a]	9.3	7.1	5.8	9.5	8.5	5.8	4.5	10.0	7.9	11.7	7.6	12.1	14.6	9.9	5.9	7.4	5.7	8.43
20:2ω6	0.3	0.3	0.7	0.2	0.3	0.5	0.3	0.3	0.2	0.2	0.3	0.3	0.1	0.3	0.4	0.3	0.3	0.31
20:4ω6	0.9	2.0	2.8	0.8	0.7	1.3	1.5	1.7	1.8	0.7	1.4	0.8	0.6	1.1	1.4	1.0	1.5	1.29
20:4ω3	0.4	0.3	0.4	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.5	0.4	0.5	0.5	0.6	0.38
20:5ω3	8.8	13.6	11.9	11.0	11.0	11.2	13.0	14.4	12.3	10.4	12.3	10.6	9.2	9.3	11.9	10.9	11.1	11.35
21:5ω2 ?	0.3	0.2	0.3	0.3	0.2	0.1	0.4	0.2	0.1	0.2	0.3	0.2	0.4	0.2	0.2	0.3	0.5	0.26
22:1ω11[a]	8.1	4.4	2.0	7.5	6.5	3.8	2.0	5.8	6.2	9.1	4.0	10.3	12.3	7.0	3.7	5.2	1.8	5.86
22:4ω6	0.1	0.1	0.4	0.1	0.1	0.2	0.4	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.3	0.1	0.4	0.20
22:5ω6	0.3	0.2	0.5	0.1	0.5	0.5	0.3	0.4	0.2	0.1	0.1	0.1	0.1	0.4	0.2	0.6	1.0	0.33
22:5ω3	1.1	1.7	3.0	1.3	1.7	2.0	1.6	1.5	1.3	1.2	1.1	0.8	0.9	1.0	2.2	1.7	2.4	1.56
22:6ω3	16.2	14.4	12.9	14.1	11.9	18.0	14.1	10.1	8.6	10.9	8.9	7.9	8.3	12.1	14.6	14.8	14.3	12.48
24:1ω9[a]	0.8	0.8	0.7	0.5	0.7	0.2	0.5	0.8	0.6	0.5	0.7	0.7	0.2	0.9	0.6	0.7	1.0	0.64
Oil content, %	56	42	31	28	40	30	15	25	42	47	54	58	67	40	42	22	32	
Iodine value	164	179	166	171	162	175	174	164	153	149	166	146	141	163	176	169	160	163

[a] For this and subsequent tables: several isomers may be present.

TABLE II. Component fatty acids of cod liver oils (four male fish pooled monthly; weight %).

Fatty acid	1962		1963												1964			Average
	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
14:0	2.9	2.7	2.0	4.2	2.6	4.6	3.3	3.1	2.1	1.7	3.2	2.7	4.8	3.1	3.7	3.9	3.6	3.19
14:1ω5[a]	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.3	0.1	0.2	0.3	0.17
15:0	0.3	0.3	0.4	0.2	0.4	0.3	0.5	0.4	0.4	0.4	0.4	0.3	0.2	0.8	0.4	0.5	0.4	0.39
16:0	14.0	12.9	18.8	11.0	13.6	14.1	13.8	13.7	15.9	13.0	13.4	13.6	12.4	13.8	12.6	12.7	12.8	13.46
16:1ω7[a]	9.0	7.2	6.8	8.7	11.9	8.4	9.8	11.4	9.1	8.1	11.5	10.4	11.1	11.5	9.3	9.4	11.5	9.76
16:2ω4	0.4	0.4	0.2	0.5	0.4	0.5	0.5	0.5	0.5	0.3	0.5	0.3	0.6	0.8	0.6	0.5	0.6	0.48
16:3ω4	0.1	0.3	0.1	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.3	0.2	0.5	0.2	0.1	0.1	0.3	0.22
17:0	0.6	0.9	0.4	0.5	0.8	1.1	0.9	0.7	0.5	0.8	0.5	0.8	0.6	1.0	0.7	0.8	0.9	0.76
17:1ω8[a]	0.6	0.5	0.5	0.3	0.6	0.4	0.5	0.6	0.7	0.6	0.5	0.6	0.3	0.8	0.4	0.5	0.5	0.52
18:0	3.2	4.0	3.3	1.7	2.1	1.8	2.3	2.2	3.4	4.5	2.5	3.1	2.0	3.5	2.3	2.8	2.1	2.74
18:1ω9[a]	23.1	22.1	25.2	18.7	29.8	23.7	25.9	25.8	29.1	26.0	22.2	24.9	18.9	22.7	22.7	21.2	21.6	23.67
18:2ω6[a]	1.0	1.3	0.8	1.1	1.2	1.4	1.1	1.7	1.6	1.2	1.4	1.4	1.6	1.5	1.5	1.9	1.3	1.38
18:3ω3	0.4	0.7	0.4	0.4	0.3	0.6	0.9	0.5	0.8	0.6	0.4	0.5	0.7	0.4	0.6	1.1	0.4	0.58
18:4ω3	0.8	1.1	0.6	0.5	0.6	0.7	1.1	0.8	0.9	1.1	1.0	0.9	1.7	0.8	0.6	1.6	0.9	0.94
19:1ω10[a]	0.1	0.3	0.4	0.1	0.1	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.23
20:1ω9[a]	7.2	6.0	5.3	14.2	7.0	10.1	6.3	4.0	4.2	5.0	9.5	6.8	10.8	4.3	7.5	7.8	10.2	7.42
20:2ω6	0.1	0.4	0.6	0.3	0.2	0.2	0.2	0.4	0.6	0.4	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.32
20:4ω6	1.3	2.0	2.5	0.7	0.9	0.8	1.5	2.4	3.5	2.2	1.2	1.7	0.5	1.6	1.4	1.2	1.1	1.55
20:4ω3	0.5	0.4	0.4	0.2	0.3	0.2	0.3	0.1	0.4	0.4	0.3	0.4	0.4	0.5	0.6	0.6	0.2	0.37
20:5ω3	11.1	11.9	11.7	7.9	10.1	8.5	12.7	5.9	13.0	13.8	12.3	15.1	10.8	12.3	12.9	11.1	8.5	11.15
21:5ω2 ?	0.3	0.3	0.1	0.3	0.2	0.1	0.2	0.7	0.2	0.1	0.2	0.2	0.4	0.2	0.2	0.2	0.3	0.26
22:1ω11[a]	6.1	4.4	1.7	13.1	4.6	8.5	2.4	0.8	1.9	2.1	7.8	1.8	9.9	1.7	6.2	5.4	8.1	5.09
22:4ω6	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.5	0.5	0.2	0.1	0.1	0.1	0.5	0.1	0.1	0.4	0.24
22:5ω6	0.4	0.4	0.4	0.3	0.4	0.6	0.4	0.6	0.4	0.1	0.2	0.3	0.3	0.8	0.3	0.4	0.7	0.42
22:5ω3	1.6	1.4	2.8	1.4	1.6	1.3	1.8	2.6	1.7	1.4	1.0	1.5	0.9	2.6	1.7	1.6	1.8	1.7
22:6ω3	14.7	17.3	13.8	12.1	9.1	11.9	13.4	19.2	7.6	15.0	8.7	11.5	10.1	13.7	12.7	14.0	10.2	12.57
24:1ω9[a]	0.6	0.6	0.4	1.0	0.5	0.2	0.2	0.9	0.5	0.7	0.4	0.5	0.7	0.3	0.8	0.5	0.9	0.57
Oil content, %	42	41	25	37	26	32	22	23	41	50	50	53	59	20	26	29	26	
Iodine value	166	185	166	147	155	152	174	159	155	186	158	167	159	175	169	157	146	163

16:1 and 18:1, did not vary nearly as much. The relationships were not so obvious for intermediate values of liver oil content or for the male fish.

The iodine values fluctuated between 141 and 186 with an average of 163 for both sexes. No clear seasonal trend could be detected, although there was a tendency for a low iodine value when the proportions of 20:1 and 22:1 were high (August–November 1963). By plotting the total percentage monounsaturated and polyunsaturated fatty acids against iodine values, an inverse relationship could be found (Fig. 1). This relationship was also pointed out by DeWitt, although this author stated that the highest iodine values were found when the fish were at their fattest in fall and winter and reached a minimum in the spring after spawning. Our results, for a localized population, indicated that the minimum was reached in August–November when the fish were the fattest and the maximum in the spring after spawning when the livers were the leanest. This contradiction may arise from the different areas and cod populations included in DeWitt's study.

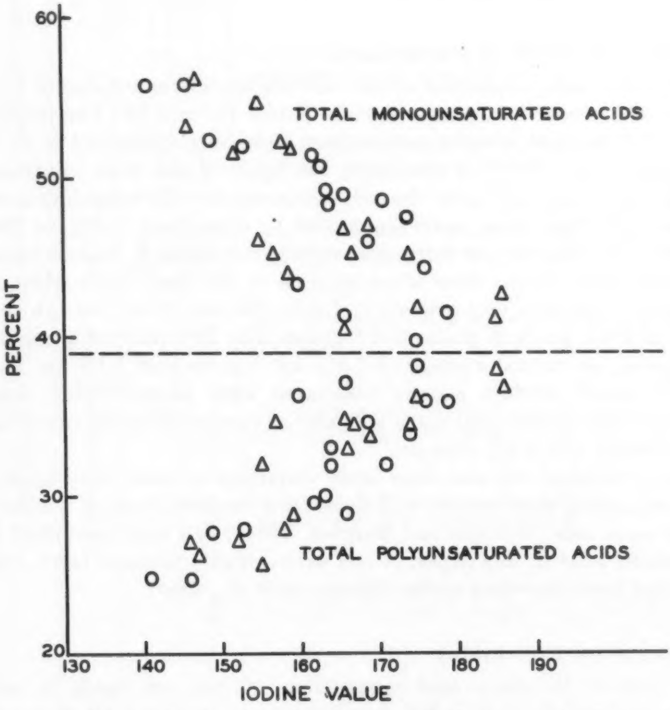

FIG. 1. Relationship between iodine values and the content of mono- and polyunsaturated acids in cod liver oil; △ = female; ○ = male fish.

Only the changes in the mono- and polyunsaturated acids seemed to have an effect on the iodine values. The saturated acids were remarkably constant over the year, rarely deviating more than 10% from the averages. Ackman and Eaton (1966) suggested a slight increase in saturated acids with iodine value increase in herring oils and discussed the correlation between iodine values, monounsaturated and polyunsaturated fatty acids, and also the ratios of the metabolically active fatty acids.

The individual polyunsaturated acids varied irregularly throughout the year in both male and female fish. A slightly lower content of 20:4ω6, 20:5, and especially 22:6 could sometimes be found when the oil content of the liver was high.

The contents of 18:4 in Nova Scotian cod liver oil were definitely lower than in oils from other areas of the North Atlantic as discussed by Lambertsen and Braekkan (1965). Their average for nine analyses from different laboratories was 2.29% whereas in our results from Tables I and II the highest monthly value was 1.4%. The average for 34 determinations of male and female fish was 0.97%. These low values could also be found in the analysis of individual fish which will be discussed later.

SEASONAL VARIATION IN FLESH LIPIDS

The fatty acid composition of the cod flesh lipids over a period of 12 months for male and female fish is tabulated in Tables III and IV. The phospholipid content of these lipid samples ranged from 80 to 86% (Jangaard et al. ,1967).

Lovern et al. (1959) in discussing the lipids of cod flesh mentioned that the triglycerides were probably the only components to show significant seasonal variation, and that these never amounted to more than 2–3% of the total. Since 20:1 and 22:1 are the fatty acids chiefly deposited in triglycerides of the liver, these acids should show some increase in the flesh lipids when the fish were fattest. This is in fact evident in Tables III and IV for the fish caught in November 1963, for both males and females. The 20:1 reached a maximum of 2.8% against an annual average of 1.6% for females and 1.1% for males. In the same month 20:4ω6, usually associated with phospholipids, reached a minimum, 2.6% (male) and 3.1% (female) as compared to the annual average of 5.1% (male) and 4.4% (female).

That individual fish can show large variations in some fatty acids can be seen by comparing these results with those from the flesh lipids of a male codfish from the same area (Ackman and Burgher, 1964a) and with individual mature fish discussed later in this paper. A cod with a highly unusual fatty acid composition has been described earlier (Jangaard et al., 1966).

FATTY ACIDS OF ROE LIPIDS

In Table V the fatty acid composition of cod roe lipids is arranged in order of increasing size of the roes relative to the fish. All the roes from March 23, 1964, were classified as ripe or ripening.

TABLE III. Fatty acid composition of cod flesh lipids (females, pooled samples; weight %).

Fatty acid	Argon ionization detector					Flame ionization detector							Average
	1963									1964			
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
14:0	0.9	0.8	1.2	1.3	0.7	0.9	1.3	1.2	0.9	0.8	0.8	0.8	0.99
15:0	0.1	0.2	0.1	0.1	0.1	0.1	0.3	0.2	0.2	0.2	0.3	0.2	0.21
16:0	19.6	21.1	20.1	20.8	(27.1)	17.7	20.3	20.3	21.1	19.8	20.7	21.1	20.21
16:1ω7[a]	2.4	2.0	2.0	2.1	2.2	2.0	3.0	2.4	1.8	2.1	1.8	2.5	2.19
18:0	3.5	3.3	3.6	3.3	3.2	4.0	3.5	3.5	3.0	3.4	3.6	3.2	3.43
18:1ω9[a]	11.0	12.3	10.8	11.1	12.5	12.0	13.2	10.9	11.4	12.0	11.7	12.0	11.76
18:2ω6	0.6	0.8	0.6	0.5	0.6	0.7	0.8	0.9	0.8	0.6	0.7	0.7	0.69
20:1ω9[a]	0.9	1.1	1.7	1.4	2.5	0.9	2.6	2.8	1.2	1.0	1.2	1.0	1.63
20:4ω6	5.0	4.2	5.6	4.4	3.7	6.5	4.0	3.1	4.6	4.9	3.7	3.7	4.42
20:4ω3	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.14
20:5ω3	16.5	17.0	16.1	16.6	14.2	17.0	18.7	17.3	16.9	15.6	17.9	15.4	16.57
22:1ω11[a]	0.7	0.9	0.9	0.7	0.8	0.5	1.1	1.1	1.0	1.1	0.5	0.4	0.81
22:5ω3	1.5	1.8	1.9	1.6	1.6	2.2	1.7	1.3	1.5	2.0	1.4	1.3	1.65
22:6ω3	33.6	31.9	33.1	34.7	30.6	33.6	29.8	33.4	33.4	34.4	32.0	36.2	33.12
Others	3.6	2.5	2.2	1.3	–	1.8	–	1.5	2.0	1.9	3.6	1.1	1.74
Iodine value	245	240	237	232	224	222	255	247	260	251	249	246	243

TABLE IV. Fatty acid composition of cod flesh lipids (males, pooled samples; weight %).

Fatty acid	Argon ionization detector					Flame ionization detector							
	1963									1964			
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Average
14:0	0.8	1.0	1.2	1.0	1.3	1.2	0.8	1.3	0.8	0.7	0.9	1.0	1.02
15:0	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.3	0.4	0.4	0.2	0.25
16:0	20.5	21.5	20.7	21.7	23.1	19.3	20.7	20.0	20.7	20.9	21.7	20.0	20.88
16:1ω7[a]	1.8	2.2	1.8	2.4	1.3	3.1	2.1	2.5	2.4	1.6	2.0	2.3	2.15
18:0	3.3	3.5	3.2	3.6	3.4	3.7	3.1	3.5	2.9	3.7	3.7	3.1	3.39
18:1ω9[a]	12.6	11.4	13.3	13.1	11.5	12.1	11.3	10.7	11.7	11.5	13.0	11.7	12.00
18:2ω6	0.5	0.7	0.4	0.6	0.3	0.5	0.6	0.9	0.5	0.7	0.7	0.6	0.58
20:1ω9[a]	1.2	0.9	1.0	0.8	0.5	1.4	1.1	2.8	0.5	0.8	1.0	1.0	1.09
20:4ω6	6.2	5.6	6.5	6.9	4.5	5.4	6.3	2.6	5.5	4.3	4.4	4.3	5.07
20:4ω3	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.3	0.1	0.1	0.2	0.2	0.16
20:5ω3	16.5	17.0	14.9	18.0	15.9	17.1	19.0	16.6	16.6	16.3	15.4	16.7	16.65
22:1ω11[a]	0.2	0.3	0.6	0.4	0.3	0.2	0.6	0.9	0.5	0.2	0.4	0.3	0.44
22:5ω3	1.5	1.2	1.6	1.9	1.7	1.8	1.8	1.5	1.7	0.7	1.6	1.8	1.61
22:6ω3	34.2	31.6	32.4	27.5	34.7	31.1	30.6	33.7	34.1	36.8	32.9	33.8	32.76
Others	0.4	2.7	2.0	1.8	1.2	2.7	1.7	2.4	1.7	1.3	1.7	2.0	1.95
Iodine value	246	237	223	239	231	222	248	240	244	258	247	245	240

TABLE V. Fatty acid composition of cod roe lipids arranged in order of increasing maturity (weight %).

	1963				1964						
Fatty acid	No. 6, 10, 11, 13 Immature Dec.	No. 11 Oct.	No. 2 Sept.	No. 21 Nov.	No. 20 Mar. 23	No. 4 Mar. 23	No. 17 Mar. 23	No. 2 Mar. 23	No. 9 Mar. 23	Average	Ackman and Burgher (1964c)
14:0	1.8	2.2	1.1	2.7	1.7	2.2	2.1	1.4	1.7	1.88	0.6
15:0	0.4	0.4	0.1	0.3	0.5	0.5	0.3	0.4	0.4	0.37	0.1
16:0	22.2	22.2	19.7	18.7	21.1	19.0	19.5	18.1	20.4	20.10	23.4
16:1ω7[a]	5.4	8.2	5.7	7.5	5.3	6.2	7.0	5.1	6.2	6.29	6.4
16:2ω4	0.7	0.2	0.1	0.3	0.1	0.4	0.1	0.1	0.6	0.29	0.1
16:3ω4	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.6	0.19	Trace
17:0	1.0	0.6	0.5	1.0	0.5	0.4	0.7	0.4	0.3	0.60	–
17:1ω8[a]	0.2	0.1	0.1	0.2	0.4	0.2	0.3	0.5	0.2	0.24	0.1
18:0	3.6	1.3	3.7	1.6	2.2	1.7	1.9	2.4	1.6	2.22	1.3
18:1ω9[a]	14.8	14.5	13.1	13.6	15.1	16.5	14.6	18.9	16.3	15.27	23.4
18:2ω6	0.7	0.5	0.4	0.7	1.1	0.9	0.6	0.7	0.8	0.70	0.2
18:3ω3	0.1	0.1	0.3	0.1	0.4	0.2	0.1	0.3	0.2	0.20	0.1
18:4ω3	0.3	0.3	0.3	0.5	0.4	0.5	0.3	0.2	0.5	0.37	0.1
19:1ω10[a]	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.2	0.3	0.16	0.1
20:1ω9[a]	2.0	2.8	2.7	3.5	2.7	2.5	4.3	1.4	2.2	2.68	1.0
20:2ω6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.13	Trace
20:4ω6	6.7	3.8	4.9	2.0	1.6	2.0	2.0	5.5	2.0	3.39	2.4
20:4ω3	0.1	0.1	0.1	0.3	0.4	0.3	0.2	0.3	0.4	0.24	0.1
20:5ω3	16.0	15.0	20.1	16.5	17.3	18.0	18.5	14.5	17.6	17.06	16.5
22:1ω11[a]	1.0	2.0	1.1	2.0	0.9	0.9	0.9	1.0	0.5	1.14	0.2
22:5ω6	0.1	0.1	0.1	0.4	0.3	0.1	0.1	0.3	0.2	0.19	0.1
22:5ω3	1.9	1.7	0.9	1.5	0.9	1.3	1.9	3.3	1.8	1.69	0.7
22:6ω3	20.0	23.0	21.1	22.7	26.9	25.4	24.4	22.9	24.7	23.46	22.1
24:1ω9[a]	1.5	1.0	3.4	1.1	0.5	0.4	0.5	0.7	0.6	1.08	0.8
Roe weight as % of fish	Avg 0.3	0.7	0.9	1.9	8.9	9.4	18.7	25.4	26.7		3.5
Weight of fish (g)	" 1660	4000	3490	7070	2330	3180	5950	3460	2920		
Length of fish (cm)	" 57	71	73	87	63	73	86	67	62		
Weight roe (g)	" 5.0	28	31	131	206	297	1113	877	781		
Weight liver (g)	" 31	262	71	628	118	55	346	65	66		
Liver weight as % of fish weight	" 1.9	6.5	2.0	8.9	8.8	1.7	5.8	1.9	2.3		
% lipid in roe	" 0.8	1.2	1.1	1.3	3.2	1.5	2.1	1.8	1.0	1.56	1.4
Unsaponifiable matter, %	" 15.0	7.5	–	8.9	20.6	9.9	8.8	8.2	11.0	11.2	13.6
Iodine value of methyl esters	" 210	195	193	206	–	213	183	–	213	201.8	200
% lipid in flesh	–	0.76	0.67	0.71	–	–	–	–	–	–	–

No definite correlation could be established between the development of the roes and the fatty acid composition of the lipids. The deviations from the average were remarkably small considering the varying stages of development of the roes.

The roe lipids were intermediate in iodine values to the female flesh and liver lipids, ranging from 183 to 213 with an average of 202 as compared to 141 to 179 (average 163) for the liver and 222 to 260 (average 243) for the flesh. Some of the fatty acids were also present in intermediate amounts; for instance the content of 22:6 averaged 23.5% as compared to 33.1% in the flesh and 12.5% in the liver. The 18:1 averaged 15.3% as compared to 11.8% in flesh and 22.9% in liver, the 14:0 was 1.9% as compared to 1.0 and 3.3%, and 16:1 was 6.3% vs. 2.2% and 9.4%. The contents of 16:0, 20:5, and 22:5ω3 were very close to the values for the flesh. Since salmon egg oil has been reported to contain up to 40% phospholipid (Jones et al., 1948), the intermediate composition of cod roe lipids between triglyceride (liver oil) and phospholipid (flesh) composition is most likely due to the presence of both types in roe lipids.

The lipid content did not vary with size or development of the roe, the values ranging from 0.8 to 3.2%. The unsaponifiable matter was quite variable, from 7.5 to 20.6% but again no correlation could be found with maturity.

FATTY ACIDS OF MILT LIPIDS

The fatty acid composition of the lipids from the male gonads (or milt) is tabulated in Table VI. No definite relationship between acid composition and maturity was observed but most of the male gonads examined were at a fairly late stage of development.

The milt lipid fatty acid composition was very close to the composition of the roe. The chief differences seemed to be in the 16:1 which was about one-third of the value found in roe, the 18:1 which was higher, and the 20:5 which was slightly lower. The other major acids, 16:0 and 22:6, were almost identical. The content of 24:1 and 14:0 was lower in the milt lipid than in the roe.

The percentage lipids in the milt was similar to values found for the roe, ranging from 0.9% to 2.3%. The unsaponifiable matter was much higher, from 12% to 28% with an average value of about 20%.

The milt from fish No. 3 caught in February 1964, had a rather unusual fatty acid composition with 18:1 29% higher than the average and 22:6 30% lower; otherwise the fatty acid composition was quite normal. The flesh lipids from this fish (Table VIII) had a fairly low content of 18:1, about 20% below the average, but otherwise an average composition.

FLESH AND LIVER LIPID COMPOSITION OF LARGE COD

In Tables VII and VIII the fatty acid composition of flesh lipids and liver oils from several single fish and also from some pooled, large, trawler-caught fish from the same area is summarized. It is at once obvious that the variations in individual fatty acids from fish to fish can be quite large.

TABLE VI. Fatty acid composition of cod milt lipids (weight %).

Fatty acid	1963 No. 20 Nov.	1964 No. 4 Jan.	No. 3 Feb.	No. 8 March 23	No. 18 March 23	No. 14 March 23	No. 16 March 23	Average
14:0	1.5	0.8	0.9	0.3	0.9	1.3	1.0	0.96
15:0	0.3	0.3	0.4	0.2	0.5	0.5	0.5	0.38
16:0	18.8	20.2	24.1	20.7	22.4	20.1	20.4	20.95
16:1 ω 7[a]	2.4	1.8	2.0	1.0	2.9	2.7	1.8	2.08
17:0	1.3	1.4	1.1	0.7	0.8	1.1	1.0	1.05
17:1 ω 8	0.1	0.3	0.2	0.1	0.5	0.3	0.4	0.27
18:0	3.1	3.4	3.2	5.0	3.8	4.0	3.7	3.74
18:1 ω 9[a]	21.4	20.8	27.3	22.2	19.5	18.6	18.4	21.17
18:2 ω 6	0.6	0.5	0.3	0.4	0.6	0.4	0.4	0.46
20:1 ω 9[a]	5.1	2.8	2.6	2.2	1.9	4.2	2.9	3.10
20:4 ω 6	1.7	5.9	7.0	6.7	8.3	3.4	3.8	5.26
20:4 ω 3	0.3	0.4	0.1	0.1	0.3	0.3	0.2	0.24
20:5 ω 3	14.5	13.0	11.0	12.4	13.4	16.8	14.5	13.66
22:1 ω 11[a]	1.4	0.5	0.4	0.4	0.3	0.6	0.7	0.61
22:4 ω 6	0.1	0.3	0.5	0.2	0.8	0.3	0.4	0.37
22:5 ω 6	0.3	0.4	0.5	0.4	0.4	0.5	0.5	0.43
22:5 ω 3	1.7	1.7	1.5	2.1	2.8	2.0	2.8	2.09
22:6 ω 3	24.1	24.7	15.5	24.3	18.5	22.0	25.0	22.02
24:1 ω 9[a]	0.4	0.5	0.3	0.7	0.5	0.3	0.4	0.44
Others	0.8	0.2	0.4	–	0.6	0.2	0.9	0.72
Milt weight as % of fish	8.1	4.3	5.9	4.5	5.0	9.5	5.4	
Weight of fish (g)	8480	2580	2520	2890	1930	2470	2300	
Length of fish (cm)	88	65	65	65	59	64	65	
Weight milt (g)	690	113	150	131	96	235	123	
Weight liver (g)	447	52	23	64	20	46	26	
Liver weight as % of fish weight	5.3	2.0	0.9	2.2	1.0	1.9	1.1	
% lipid in milt	0.9	2.3	1.8	1.9	1.9	1.8	1.2	1.69
Unsaponifiable in lipid (%)	12.1	–	21.8	21.3	27.1	17.6	19.1	19.8
Iodine value of methyl esters	204	197	171	203	182	201	207	195
% lipid in flesh	–	0.57	0.64	–	–	–	–	–

In Table VII the fatty acid composition of the liver lipids of individual mature fish is given. The long-chain monoethylenic acids 20:1 and 22:1 were considerably higher then in the monthly samples and the average value for 20:1 (10.8%) was closer to Lambertsen and Braekkan's (1965) average (11.3%) than to that of this study (7.8%). Of other major acids, the 16:0 was quite

TABLE VII. Fatty acid composition of cod liver oils (large fish; weight %).

Fatty acid	1963				1964					Ackman and Burgher (1964b)	Lambertsen and Braekkan (1965) Average	Average Tables I and II
	No. 2 Sept. F	No. 11 Oct. F	No. 21 Nov. F	No. 20 Nov. M	No. 4 Jan. M	No. 3 Feb. M	Pooled Mar. 23 FX[b]	Pooled Mar. 23 MX	No. 14 Mar. 23 M			
14:0	4.5	4.2	5.3	3.9	3.0	3.7	3.8	3.6	4.0	3.5	3.24	3.3
14:1ω5[a]	0.3	0.3	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.2		0.2
15:0	0.4	0.5	0.6	0.4	0.3	0.7	0.4	0.5	0.4	0.5		0.4
16:0	12.7	12.2	13.6	11.6	12.1	11.0	11.2	10.2	10.0	10.4	10.78	13.4
16:1ω7[a]	12.1	10.7	12.1	10.2	6.0	9.5	12.3	10.6	10.5	12.2	8.92	9.6
16:2ω4	0.8	0.6	0.8	0.6	0.4	0.6	0.6	0.5	0.3	0.9		0.5
16:3ω4	0.4	0.5	0.3	0.4	0.1	0.2	0.6	0.5	0.3	0.2		0.2
17:0	1.0	0.8	0.5	0.6	0.7	0.8	0.8	1.1	0.7	0.1		0.8
17:1ω8[a]	0.3	0.3	0.1	0.3	0.3	0.6	0.4	0.5	0.4	0.2		0.5
18:0	1.8	2.6	1.7	1.8	3.6	2.1	2.2	2.6	2.0	1.2	2.49	2.7
18:1ω9[a]	19.1	23.3	26.8	21.0	19.7	19.6	20.7	22.5	22.7	19.6	23.90	23.4
18:2ω6	1.4	1.5	1.4	1.3	1.8	1.2	1.5	1.9	1.7	0.8	1.97	1.4
18:3ω3	0.5	0.7	0.9	0.8	1.0	0.4	0.5	0.7	0.5	0.2	0.6	0.6
18:4ω3	1.7	1.1	2.0	1.2	1.3	0.4	1.2	1.3	1.8	0.7	2.29	1.0
19:1ω10[a]	Trace	0.2	Trace	Trace	0.2	0.3	0.2	0.2	Trace	0.1		0.2
20:1ω9[a]	10.2	11.8	7.2	12.1	11.1	11.4	12.8	8.7	9.8	14.6	11.34	7.8
20:2ω6	0.1	0.2	0.3	0.1	0.4	0.4	0.3	0.3	0.2	0.2		0.3
20:4ω6	0.4	0.5	0.6	0.5	1.2	2.0	0.5	1.4	0.9	1.2		1.4
20:4ω3	0.3	0.3	0.6	0.4	0.4	0.5	0.5	0.5	0.5	0.5		0.4
20:5ω3	8.9	9.3	11.8	9.8	8.9	7.8	8.3	13.0	13.9	5.0	9.01	11.5
21:5ω2 ?	0.3	0.4	0.6	0.3	0.2	0.1	0.3	0.4	0.5	0.2		0.3
22:1ω11[a]	9.4	10.8	5.3	10.7	10.0	9.7	9.6	4.2	5.4	13.3	5.62	5.3
22:5ω6	0.2	0.1	0.1	0.2	0.6	1.2	0.3	0.5	0.2	0.1		0.3
22:5ω3	1.8	0.9	1.1	1.0	2.6	3.0	1.7	1.9	1.6	1.9		1.6
22:6ω3	11.5	7.1	5.9	10.0	17.0	12.3	9.0	11.3	11.4	10.5	10.43	12.5
24:1ω9[a]	–	0.4	0.5	0.6	0.2	0.3	0.3	0.5	0.4	0.7		0.6
Iodine value	151	142	150	173	176	151	141	167	164	155		163

[b]X = fish caught with bottom trawl.

TABLE VIII. Fatty acid composition of cod flesh lipids (large fish; weight %).

Fatty acid	1963		1964							
	No. 11 Oct. F	No. 21 Nov. F	No. 4 Jan. M	No. 3 Feb. M	Pooled Feb. 24 FX	Pooled Feb. 24 MX	Pooled Mar. 23 FX	Pooled Mar. 23 MX	Ackman and Burgher (1964a)	Average Tables III and IV
14:0	1.6	2.0	0.9	0.7	1.1	1.2	0.9	1.2	0.4	1.0
15:0	0.2	0.1	0.3	0.2	0.3	0.3	0.3	0.4	0.1	0.2
16:0	18.5	18.1	19.7	20.4	24.2	25.9	18.4	18.4	24.1	20.5
16:1ω7[a]	3.8	3.5	1.5	1.5	2.5	3.7	3.0	3.0	1.1	2.2
18:0	3.1	3.6	4.5	4.9	4.1	4.6	3.9	4.0	3.4	3.4
18:1ω9[a]	13.1	13.2	11.5	9.5	15.1	18.9	15.0	14.3	7.8	11.9
18:2ω6	1.0	1.4	0.7	0.5	0.6	0.6	1.2	0.9	0.3	0.6
18:4ω3	0.6	0.5	0.2	0.1	0.1	0.1	0.3	0.3	0.1	–
20:1ω9[a]	3.3	3.7	0.6	1.2	1.7	1.6	1.9	1.7	1.2	1.4
20:4ω6	3.2	2.7	9.0	8.9	6.8	5.2	6.0	3.8	3.5	4.7
20:4ω3	0.3	0.5	0.2	0.3	0.1	0.1	0.4	0.4	0.2	0.2
20:5ω3	18.6	18.0	15.6	18.9	15.9	14.2	21.7	16.1	17.6	16.6
22:1ω11[a]	1.1	1.4	0.9	0.5	0.4	0.2	0.4	0.6	1.1	0.6
22:5ω3	1.7	1.5	2.5	1.4	2.3	1.7	2.5	1.6	0.6	1.6
22:6ω3	27.9	27.8	30.8	29.2	22.7	19.9	22.1	31.8	37.5	32.9
Others	2.0	2.0	1.1	1.8	2.1	1.8	2.0	1.5	–	1.8
Iodine value	239	230	249	249	212	185	231	240	248	241

constant at 10–13%, the 18:1 with one exception (26.8%) was present in amounts of 19.1% to 23.3% but again we found wider variations in the long-chain, highly unsaturated acids, especially 22:6.

From Table VIII, we can again see that in the flesh lipids 16:0 was fairly constant whereas 18:1 showed considerable variation, ranging from 9.5% to 18.9%. The highest values were found in pooled, very large fish. Average weights for the female fish were 9500 g, for the males 8090 g, caught by bottom trawl in the same area (MX, FX, February 24, 1964). These two samples also had the lowest content of 22:6, namely 19.9% and 22.7% as compared with the average of 33%. Their lipid content and non-phospholipid content was similar to the average monthly samples and the iodine values of the flesh fatty acids were low, respectively 185 and 212, (normally 220–250). Another sample of large, pooled, female fish, from March 23, 1964, also had a low content of 22:6 (22.1%), but had a compensating high value for 20:5 (21.7%) to give an iodine value (231) near the seasonal average.

Arachidonic acid, 20:4ω6, also varied quite widely in the flesh lipids of individual fish, from 2.7% to 9.0%. In the two fish with the highest values, 8.9 and 9.0%, the percentages of 20:1, 18:1, and 16:1 were low with normal levels of the other polyunsaturated acids. The liver condition indicated that these fish were quite lean.

In an earlier communication we discussed the highly unusual fatty acid composition of a single cod having over 50% 18:1 in the liver oil (Jangaard et al., 1966). From the results in Tables VII and VIII it is obvious that large variations can also occur even in individual "normal" fish, and that a single analysis can, in some cases, give quite misleading results for the fatty acid compositions.

REFERENCES

ACKMAN, R. G., AND R. D. BURGHER. 1964a. Cod flesh: Component fatty acids as determined by gas–liquid chromatography. J. Fish. Res. Bd. Canada, **21**(2): 367–371.

1964b. Cod liver oil: Component fatty acids as determined by gas–liquid chromatography. Ibid., **21**(2): 319–326.

1964c. Cod roe: Component fatty acids as determined by gas–liquid chromatography. Ibid., **21**(3): 469–476.

1965. Cod liver oil fatty acids as secondary reference standards in the GLC of polyunsaturated fatty acids of animal origin: Analysis of a dermal oil of the Atlantic leatherback turtle. J. Am. Oil Chemists' Soc., **42**(1): 38–42.

ACKMAN, R. G., AND C. A. EATON. 1966. Some commercial herring oils: Fatty acid composition. J. Fish. Res. Bd. Canada, **23**(7): 991–1006.

ACKMAN, R. G., AND J. C. SIPOS. 1964. Application of specific response factors in the gas-chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. J. Am. Oil Chemists' Soc., **41**(5): 377–378.

DEWITT, K. W. 1963. Seasonal variations in cod liver oil. J. Sci. Food Agr., **14**: 92–98.

GRUGER, E. H., JR., R. W. NELSON, AND M. E. STANSBY. 1964. Fatty acid composition of oils from 21 species of marine fish, freshwater fish and shellfish. J. Am. Oil Chemists' Soc., **41**: 662–667.

JANGAARD, P. M., H. BROCKERHOFF, R. D. BURGHER, AND R. J. HOYLE. 1967. Seasonal changes in general condition and lipid content of cod from inshore waters. J. Fish. Res. Bd. Canada, **24**(3): 607–612.

JANGAARD, P. M., R. G. ACKMAN, AND J. C. SIPOS. 1966. Unusual fatty acid composition of lipids from a cod, *Gadus morhua*. Ibid., **23**(11): 1809–1810.

JONES, G. IVOR., E. J. CARRIGAN, AND J. A. DASSOW. 1948. Utilization of Alaskan Salmon cannery waste. U.S. Dept. Com., Office Tech. Serv., Rept. No. 2, Sect. 2.

LAMBERTSEN, GEORG, AND OLAF R. BRAEKKAN. 1965. The fatty acid composition of cod liver oil. Fiskeridirektorat. Skrifter, Ser. Tekn. Und. Vol. IV, No. 11. 14 p.

LOVERN, J. A., JUNE OLLEY, AND HELEN A. WATSON. 1959. Changes in the lipids of cod during storage of cod. J. Sci. Food Agr., **10**: 327–337.

OLLEY, JUNE, AND W. R. H. DUNCAN. 1965. Lipids and protein denaturation in fish muscle. Ibid., **16**(2): 99–104.

Heterogeneity of Surf Smelt, *Hypomesus pretiosus* (Girard), in the State of Washington, as Judged by Incidence of Larval *Anisakis* (Nematoda)[1,2]

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ABSTRACT

Spawning populations of surf smelt, *Hypomesus pretiosus* (Girard), from three distinct localities on the coast of Washington State revealed different levels of infestation by larval nematodes, genus *Anisakis*, in 1962. There was high incidence of the parasite among smelt from the outer coast; in contrast, incidence was low in one of the populations examined from two localities within Puget Sound, and the other was unparasitized. On the basis of the incidence of the parasite, inferences were drawn concerning the distribution, movements, and admixture of the three populations.

INTRODUCTION

The occurrence and incidence of parasites have been utilized with increasing frequency during recent years to distinguish stocks of fish. Parasitism of fishes is a common natural phenomenon, and the parasites infesting a species of fish as well as their incidence may vary between geographical areas or even between populations inhabiting the same locality. The use of larval nematodes, genus *Anisakis*, to differentiate between three spawning populations of surf smelt, *Hypomesus pretiosus* (Girard), in the state of Washington is reported herein.

According to Kabata (1963) one of the earliest applications of the technique was by the Russian parasitologists Dogiel and Bykhovskaya (1939), who identified two stocks of acipenserid fish in the Caspian Sea on the basis of their parasitic fauna. During the last 10 to 15 years accelerating attention has been focused on the use of parasites as a means of separating fish populations. Bishop and Margolis (1955) reported that the herring in British Columbia were frequently infected with *Anisakis* larvae, and found different levels of infection by area. Populations of herring and redfish in the western North Atlantic were

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identified by Sindermann (1957) on the basis of various parasites including larval nematodes (Anisakinae). Scott and Martin (1959) differentiated populations of Atlantic cod by the level of infection of nematode larvae in fillets.

Previous investigations indicate that for parasites to serve as a means of identifying fish populations, certain characteristics must be shown by the parasite-host relationship: (1) the parasite is common in one population and rare or absent in another; (2) the infestation continues over a reasonably long period of time; and (3) the incidence of infection remains relatively stable during the period of study. Other features which enhance the value of parasites as tags are that (4) the infestation does not result in large-scale mortalities; (5) the fish species studied is the sole vertebrate host in the parasite's life cycle; and (6) the parasite infests a relatively large proportion of the population. In actual application, however, all requirements are not consistently met.

MATERIALS AND METHODS

Sexually mature surf smelt were collected in Washington State in 1962 at Lapush, on the open Pacific coast, and at Utsaladdy and Hood Canal, in inner Puget Sound (Fig. 1). The samples from Lapush and Utsaladdy were taken from May to October, and those from Hood Canal from August to December, when populations of smelt approached the beaches to spawn. Three collections were made at Hood Canal, and two at both Lapush and Utsaladdy.

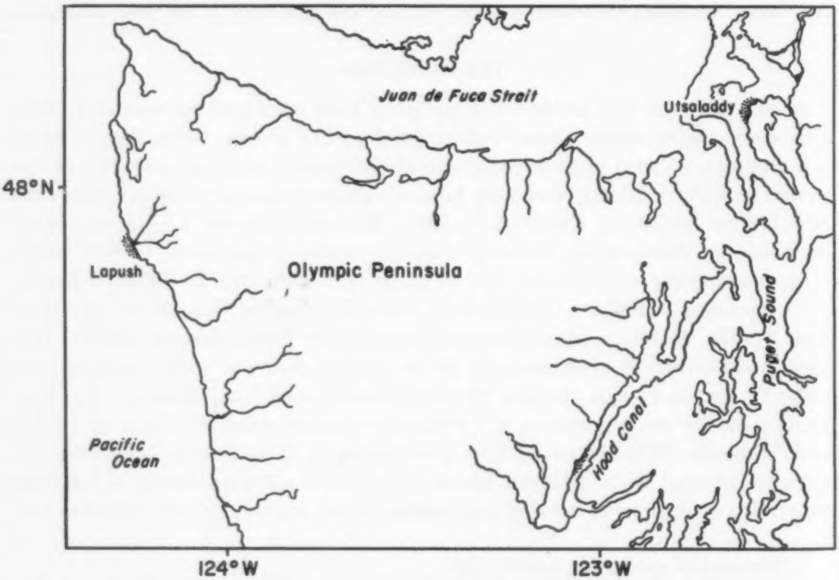

FIG. 1. Spawning localities for surf smelt at Hood Canal and Utsaladdy in Puget Sound and Lapush on the coast of Washington.

The fish were preserved in 10% formalin. From 100 to 150 fish were selected for examination from each collection to include representatives of the entire size distribution and equal numbers of males and females.

The Hood Canal smelt were mostly 1-year-olds, the Utsaladdy smelt 1-year-olds and 2-year-olds, and the Lapush population mostly 2-year-olds.

Nematodes were removed from the fish by dissection, counted, and recorded. Stained and mounted specimens were identified as larval *Anisakis* sp. The parasites occurred free in the body cavity and encysted on the liver.

DIFFERENCES IN INFESTATION

Larval nematodes were common among the smelt taken at Lapush but were rare among those taken from Hood Canal and were not found in any of those from Utsaladdy (Table I). The percentage of parasitized fish in the Lapush samples ranged from 28.6 to 54.3 (mean 44.5%), and in the Hood Canal samples from 0 to 9.8 (mean 3.5%). A X^2 analysis of the data for the Lapush samples showed no significant difference in the rates of infestation of male and female smelt ($X^2_{1\ d.f.} = 2.22$).

In the Lapush population the parasitized smelts (Table II) ranged in size from 134 to 193 mm (fork-length) and included a dominant 2-year-old group and a few 3-year-olds. Linear regression was applied to the pooled data for the two samples, all lengths and both sexes, to determine the relationship between the number of parasites and length of the fish. The null hypothesis of no relationship between intensity of infestation and length of fish was rejected below $P = 0.001$ ($t_{115} = 4.15$). However, regression analysis within the two age-groups revealed no significant relationship between intensity of infestation and fish

TABLE I. Incidence of larval *Anisakis* in surf smelt from Hood Canal, Lapush, and Utsaladdy during the 1962 spawning season.

Locality	Number of sample	Date	Sex	Number of fish examined	Number of infested fish	% infested fish
Hood Canal	1	Oct. 7	Male	71	7	9.8
			Female	69	1	1.2
	2	Oct. 20	Male	65	3	4.6
			Female	60	1	1.6
	3	Nov. 4	Male	66	0	0.0
			Female	69	2	2.8
Lapush	1	Aug. 5	Male	57	25	43.9
			Female	70	20	28.6
	2	Sept. 13	Male	70	38	54.3
			Female	66	34	51.5
Utsaladdy	1	July 29	Male	81	0	0.0
			Female	39	0	0.0
	2	Aug. 25	Male	100	0	0.0
			Female	27	0	0.0

TABLE II. Frequency distributions of larval *Anisakis* in Lapush smelt, 1962.

	Number of fish with parasites			
	Males		Females	
Number of parasites	Sample 1	Sample 2	Sample 1	Sample 2
0	32	32	50	32
1	8	9	4	9
2	4	15	5	12
3	5	6	6	3
4	5	3	2	4
5	2	2	–	2
6	–	2	1	1
7	1	–	1	–
9	–	–	1	–
12	–	–	–	1
14	–	1	–	–
15	–	–	–	1
21	–	–	–	1
Total	57	70	70	66

length (t_{94} = 0.06 for 2-year-olds, and t_{19} = 1.98 for 3-year-olds). The data indicate, therefore, that the largest or oldest smelt carried the heaviest burdens of larval nematodes.

DISCUSSION

Infestations of larval *Anisakis* served to identify the smelt populations studied. Lapush fish are distinguished from Hood Canal and Utsaladdy fish by a relatively high incidence of infestation. The parasite's incidence permits deductions concerning the general locations of the feeding grounds of the two stocks and their admixture.

It is known that marine mammals commonly serve as definitive hosts for adult *Anisakis* and that the early larval stages occur in marine crustaceans (Ouspenskaia, 1960). On the Pacific coast, adult *Anisakis* occur in finback, sperm, beaked, humpback, sei, and killer whales (Margolis, 1954; Margolis and Pike, 1955). Cetaceans are primarily inhabitants of the open ocean. Except for the harbor porpoise, the killer whale, and to a lesser extent the humpback whale, their occurrence in Puget Sound is infrequent (University of Washington, 1954). Thus, it is concluded that the infested Lapush fish had fed along the open coast.

It can also be deduced, from the virtual absence of the parasite, that the two Puget Sound populations fed in inside waters. It is presumed that seasonal phenomena in the occurrence of mammalian hosts and Utsaladdy surf smelt preclude infestation of the smelt by larval *Anisakis*.

Presence of the parasite in some Hood Canal smelt may be explained in three possible ways. They may have ingested infested crustaceans carried into Puget Sound from the outer coastal area by currents; some fish may have wandered into Juan de Fuca Strait or beyond while feeding; or infested marine mammals may have wandered into that part of the Sound frequented by Hood Canal fish.

The parasitological evidence suggests that the three spawning populations of surf smelt studied existed as distinct entities during at least part of the feeding phase of their life histories, the Lapush population along the outer coast and the Hood Canal and Utsaladdy populations in inside waters. Furthermore, the absence of *Anisakis* in the Utsaladdy smelt indicates that their feeding area differed in some degree from that of the Hood Canal fish.

Thus, heterogeneity is a feature of the surf smelt in the state of Washington. Corroborative evidence of population heterogeneity is provided by studies of meristic and morphometric characters and erythrocyte antigen composition (Kilambi, 1965; Kilambi et al., 1967).

REFERENCES

BISHOP, Y. M. M., AND L. MARGOLIS. 1955. A statistical examination of *Anisakis* larvae (Nematoda) in herring (*Clupea pallasi*) of the British Columbia coast. J. Fish. Res. Bd. Canada, **12**: 571–592.

DOGIEL, V. A., AND B. E. BYKHOVSKAYA. 1939. Parasites of fishes of the Caspian Sea (In Russian). Tr. Kompleksn. Izuch. Kasp. Morya, 7. (*Cited by* Kabata, 1963).

KABATA, Z. 1963. Parasites as biological tags. Intern. Comm. Northwest Atlantic Fish., Spec. Publ. No. 4. p. 31–37.

KILAMBI, R. V. 1965. Heterogeneity among three spawning populations of the surf smelt, *Hypomesus pretiosus* (Girard), in the State of Washington. Ph.D. Thesis. University of Washington, Seattle. 154 p.

KILAMBI, R. V., F. M. UTTER, AND A. C. DELACY. 1967. Differentiation of spawning populations of the surf smelt *Hypomesus pretiosus* (Girard) by serological methods. J. Marine Biol. Assoc. India. (In press.)

MARGOLIS, L. 1954. List of parasites recorded from sea mammals caught off the west coast of North America. J. Fish. Res. Bd. Canada, **11**: 267–283.

MARGOLIS, L., AND G. C. PIKE. 1955. Some helminth parasites of Canadian whales. Ibid., **12**: 97–120

OUSPENSKAIA, A. V. 1960. Parasitofaune des Crustacés benthiques de la mer de Barents (Exposé Preliminaire). Ann. Parasitol. Humaine Com., **35**(3): 221–242.

SCOTT, D. M., AND W. R. MARTIN. 1959. The incidence of nematodes in the fillets of small cod from Lockeport, Nova Scotia, and the southwestern Gulf of St. Lawrence. J. Fish Res. Bd. Canada, **16**: 213–221.

SINDERMANN, C. J. 1957. Diseases of fishes of the western North Atlantic. V. Parasites as indicators of herring movements. Maine Dept. Sea Shore Fish. Res. Bull. No. 27. 30 p.

UNIVERSITY OF WASHINGTON, Seattle, Washington. Dept. Oceanogr. 1954. Puget Sound and approaches, a literature survey. Vol. III. Physical oceanography, marine biology and general summary. 175 p. University of Washington, Seattle, Washington.

Some Linear, *Iso*, *Anteiso*, and Multiple-Branched Fatty Acids of Marine Origin: Analyses on Packed and Open-Tubular Gas–Liquid Chromatographic Columns with Particular Reference to Pristanic and Phytanic Acids[1]

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ABSTRACT

The analysis of esters of saturated fatty acids isolated from marine lipids, or of the saturated esters produced by hydrogenation of whole ester mixtures, must be carried out on both polar and non-polar columns to obtain adequate qualitative identification and quantitative estimation of minor saturated components. On packed columns linear odd-numbered, *Iso*, *Anteiso*, and multiple-branched acids such as pristanic (2,6,10,14-tetramethylpentadecanoic), phytanic (3,7,11,15-tetramethylhexadecanoic), and 4,8,12-trimethyltridecanoic acids can be determined. On open-tubular (capillary) columns the foregoing acids and additional minor components are more readily determined. Polyester (butanediol succinate) open-tubular columns do not show the effect of shift of retention time with relative concentration, whereas this may be a severe handicap in reliable use of silicone (SE-30) open-tubular columns. High-efficiency polyester open-tubular columns partially resolved stereoisomers of pristanic and phytanic acids.

INTRODUCTION

The presence of, and analysis for, odd-numbered linear fatty acids in marine oils is well established, and interest has recently shifted to the branched-chain fatty acids. In addition to the better-known fatty acids of the *Iso* and *Anteiso* series it was reported in a recent survey of the minor saturated fatty acids in marine lipids (Ackman and Sipos, 1965) that three fatty acids with several substituent methyl groups attached to the chain were probably present. Subsequently, three fatty acids were isolated from an unidentified marine oil (probably herring oil) and identified as 4,8,12-trimethyltridecanoic, pristanic (2,6,10,14-tetramethylpentadecanoic), and phytanic (3,7,11,15-tetramethylhexadecanoic) acids (Sen Gupta and Peters, 1966). The latter acid has recently been the subject of considerable biochemical interest and has been isolated from several terrestial animal sources (Sonneveld et al., 1962; Lough, 1963, 1964a; Hansen, 1965b, c, 1966a; Avigan, 1966a, b; Kremer, 1965; and others), and also from blue whale bone oil (Sano, 1966). The same carbon skeleton also occurs in certain bacteria (Kates et al., 1965). 2,6,10,14-Tetramethylpentadecanoic acid has also been recovered from terrestial animal sources (Hansen, 1964; Hansen and Morrison, 1964; Hansen, 1965a, 1966a; Avigan, 1966b).

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A part of the interest in branched-chain fatty acids stems from the potential conversion of marine lipids to petroleum (Leo and Parker, 1966). Cason and Graham (1965) isolated 2,6,10,14-tetramethylpentadecanoic and 3,7,11,15-tetramethylhexadecanoic acids from a California petroleum. However, in lieu of 4,8,12-trimethyltridecanoic acid they found 2,6,10-trimethylhendecanoic and 3,7,11-trimethyldodecanoic acids. Several isoprenoid fatty acids have been identified in Green River oil shale (Eglinton et al., 1966).

The hydrocarbon pristane (2,6,10,14-tetramethylpentadecane) is also found in petroleum (Bendoraitis et al., 1962) and is commonly found in marine lipids (e.g. shark liver oils, Sörensen and Mehlum, 1948; Christensen and Sörensen, 1951; herring oils, Lambertsen and Holman, 1963; Hallgren and Larsson, 1963). The occurrence of pristane and its unsaturated forms (zamenes), and of phytadiene hydrocarbons, in zooplankton has been well documented (Blumer et al., 1963, 1964; Blumer and Thomas, 1965a, b).

It is tempting to account for the presence of most of the above materials as fundamentally derivatives of phytol. Diasteomeric forms (see below) of pristanic and phytanic acids can indicate this origin (Ackman and Hansen, 1967) and influence optical activity (Hansen, 1966a). 3,7,11-Trimethyldodecenoic (farnesoic) acid is synthesized in the liver by mammals (Christophe and Popják, 1961) and has the same basic isoprene condensation skeleton. The saturated form isolated by Cason and Graham (1965) thus could be reduced farnesoic acid or could be derived by oxidation from a 2,4-phytadiene which occurs in zooplankton (Blumer and Thomas, 1965a).

4,8,12-Trimethyltridecanoic acid could be derived by oxidation from the two possible 1,3-phytadienes (Blumer and Thomas, 1965a) or from the principal "zamene" (2,6,10,14-tetramethyl-2-pentadecene) (Blumer and Thomas, 1965b).

Phytanic acid is reasonably derived from oxidation of fully saturated phytol, but pristanic acid is somewhat more difficult to account for. It could come from unspecified oxidative intermediates through the loss of one carbon atom in the conversion of phytol to phytanic acid and in the course of the same reaction pristane may be formed (cf. Sörensen and Sörensen, 1949). Alternatively, oxidation of a "zamene" (2,6,10,14-tetramethyl-1-pentadecene) would produce this material. It is of interest to note that 2,6,10,14-tetramethylpentadecanoic acid is structurally related to 3,7,11,15-tetramethylhexadecanoic acid in the same fashion as 2,6,10-trimethylhendecanoic acid is related to 3,7,11-trimethyldodecanoic acid (Cason and Graham, 1965).

This type of speculation would be more profitable if more were known about the metabolism of hydrocarbons in living organisms. Even fully saturated hydrocarbons may be oxidized (Wakabayashi and Shimazono, 1963). The occurrence of free hydrocarbons, etc., in sea water is also known (Jeffrey, 1966) and in this phase various materials could be oxidized and the breakdown products recycled into the aquatic food chain with particulate matter (Sutcliffe et al., 1963; Barber, 1966).

The isolation of both the single-branched (*Iso* and *Anteiso*) fatty acids, and the multiple-branched fatty acids, may involve several different types of preparative separations (Avigan, 1966a), although urea complexing is usually a key step (Sano, 1966; Sen Gupta and Peters, 1966; Nicolaides and Ray, 1965; Ackman et al., 1963; Grimmer and Jacob, 1965).

In straightforward gas–liquid chromatographic analyses these saturated branched-chain materials constitute an irritating "background" in the chromatograms, since in the complex C_{14}–C_{18} region (polar columns) they usually appear as baseline irregularities or shoulders on unsaturated components of interest. With the advent of high-efficiency open-tubular (capillary) columns they become of more acute interest since they may now be resolved to some extent from each other or from other materials, and also since the high-efficiency columns offer a means of determining the proportions of the various isomers without exhaustive concentration steps.

The present report is concerned with means of identifying the significant saturated minor branched-chain and normal linear odd-numbered fatty acids with packed-column or open-tubular column gas–liquid chromatographic equipment. Two types of samples will be discussed, either the saturated fatty acid esters isolated from esters from whole marine oils, or the hydrogenated esters obtained from the whole ester mixture.

EXPERIMENTAL

GAS–LIQUID CHROMATOGRAPHY

The apparatus employed in initial studies was a Wilkens/Varian Hy-FI with SE-30, EGSS-Y, and EGSS-X packed columns (the latter two materials are organosilicone copolymers of ethylene glycol, succinic acid, and methyl silicone, from Applied Science Laboratories). The operating conditions have been described elsewhere (Ackman and Sipos, 1965).

The open-tubular gas–liquid chromatographic studies were carried out with a Perkin-Elmer model 226. Columns were either butanediol-succinate (BDS) or SE-30, 150 ft in length and 0.01 inch (inside diam). Usual operating conditions were, respectively, 170 C, 40 psig helium, and 190 C, 30 psig helium. Injection port temperature was 260 C and a No. 2 inlet splitter was employed. Recordings were made on Honeywell 1-mv span recorders.

MATERIALS

The saturates isolated from the methyl esters prepared from whole oils by silver nitrate–silicic acid chromatography were those of cod liver oil and seal oil previously described (Ackman and Sipos, 1965). The triglycerides were isolated from a fresh preparation of cod liver lipids, transesterified with boron trifluoride–methanol (Morrison and Smith, 1964) and hydrogenated in a precision semi-micro hydrogenator in ethanol (Vandenheuvel, 1952; Ackman and Burgher, 1964b).

Authentic *Iso* and *Anteiso* fatty acids were purchased, as their methyl esters, from Applied Science Laboratories. A sample of 3,7,11-trimethyldodecanoic acid was obtained through the courtesy of Dr L. H. Sarett, Merck, Sharp and Dohme, New Jersey. Dr R. P. Hansen (D.S.I.R., Wellington, New Zealand) donated methyl esters of pristanic (2,6,10,14-tetramethylpentadecanoic) and phytanic (3,7,11,15-tetramethylhexadecanoic) acids of terrestial origin. Dr A. K. Sen Gupta (Unilever, Hamburg, Germany) furnished methyl esters of 4,8,12-trimethyltridecanoic, pristanic, and phytanic acids isolated from a marine fish oil.

Notation: For brevity in the figures the latter three acids are, respectively, designated A, X, and Y. The notation chain length: number of double bonds is used for esters of particular fatty acids, and I, AI, and Br prefixes in the figures refer, respectively, to *Iso*, *Anteiso*, and unresolved pairs of *Iso* and *Anteiso* fatty acid methyl esters. Equivalent chain length (Miwa et al., 1960) is abbreviated ECL and fractional equivalent chain length FCL.

RESULTS AND DISCUSSION

PACKED COLUMNS

Packed gas–liquid chromatographic columns were employed in an earlier study of the saturates isolated from various marine lipids (Ackman and Sipos, 1965). Actual gas–liquid chromatograms of methyl esters of saturated fatty acids from cod liver oil are shown in Fig. 1. With the SE-30 phase the separation of *Iso* and *Anteiso* fatty acids in the C_{15} and C_{17} chain lengths seemed curiously inadequate, although co-injection of authentic materials indicated that some separation should take place. A large component (Y) also appeared in the position likely for *Anteiso* 18:0. Analysis on an efficient polyester column of moderate polarity (EGSS-Y) gave a quite different pattern for the minor components. The *Iso* and *Anteiso* acids of the C_{15} and C_{17} chain lengths were well separated. Careful calculation of the areas of the various peaks from a number of different marine lipids, using the 15:0 and 18:0 peaks as internal standards, indicated that component Y was displaced on the EGSS-Y column to a position coincident with 17:0, and that two other components, A and X respectively, coincident in the SE-30 analysis with the *Iso* and *Anteiso* C_{15} and C_{17} materials could not be accounted for on the EGSS-Y analysis. A further analysis on a more polar liquid phase (EGSS-X) indicated additional shortening of retention time for component Y, relative to normal and methyl single-branched components, to a position between *Iso* and *Anteiso* 17:0. Moreover, a prominent peak immediately before 16:0 could be identified as component X, indicating that in the EGSS-Y analysis this component had been under the 16:0 peak. On the assumption that these three components were multiple-branched chain fatty acids with similar properties it was therefore assumed that A would be found under the 14:0 peak on the polyester analyses. In an earlier study of the urea fractionation of the whole methyl esters from seal oil (Ackman et al., 1963) the non-complexing fraction IV had in fact contained a very noticeable

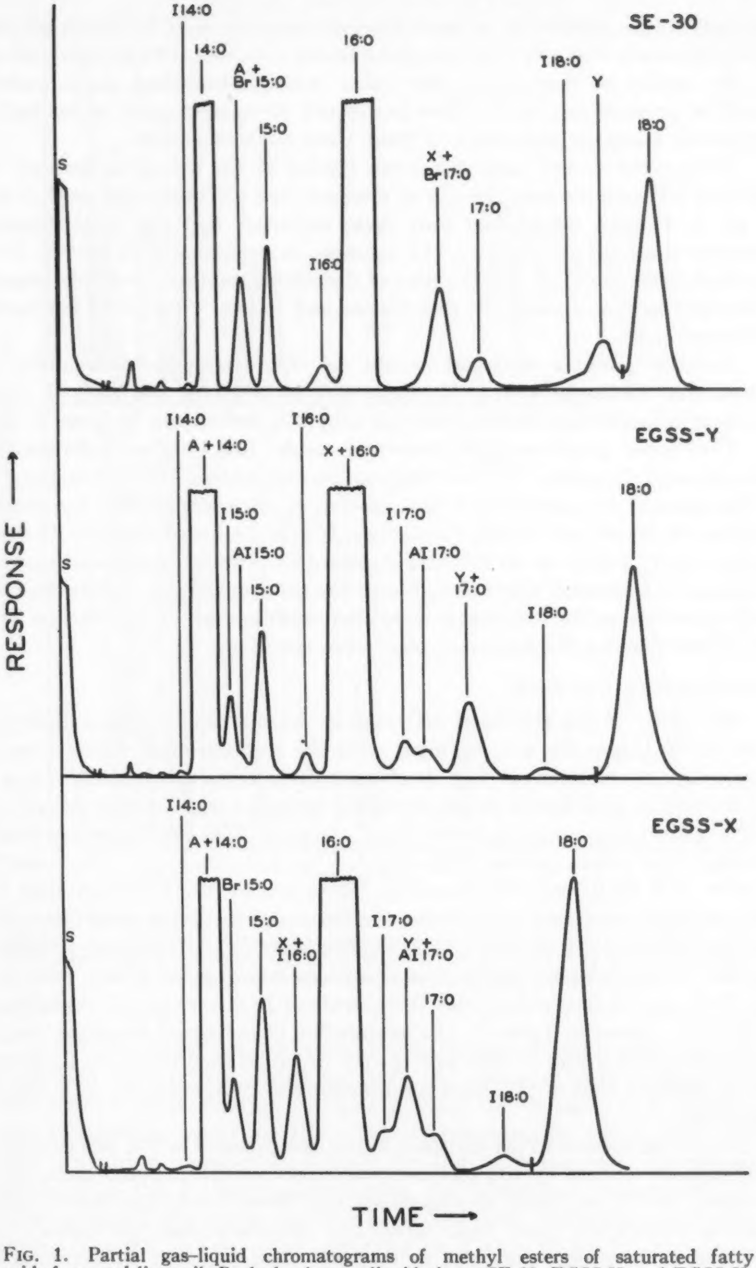

FIG. 1. Partial gas–liquid chromatograms of methyl esters of saturated fatty acids from cod liver oil. Packed columns, liquid phases SE-30, EGSS-Y, and EGSS-X. Experimental conditions and notation given in text. 18:0 at 2 X attenuation of rest of components.

component, also labelled A, in nearly the same position as 14:0 on both neopentyl glycol-succinate and ethylene glycol-succinate columns. It was also indicated by the results of that study that other multiple-branched chain materials would be present, but at that time gas–liquid chromatographic technology did not permit adequate separation of these from other materials.

Subsequent to the packed column studies of the saturates isolated from cod liver oil methyl esters the gift of pristanic and phytanic acid methyl esters by R. P. Hansen established that these materials had the same respective retention times on all three packed columns as components X and Y. It was therefore inferred that A was the ester of the other prominent multiple-branched chain fatty acid of marine oils (Sen Gupta and Peters, 1966), 4,8,12-trimethyltridecanoic acid.

Another possible material would be 3,7,11-trimethyldodecanoic acid (Cason and Graham, 1965). Although not included in the packed column studies it is known (see below) that this acid falls well before 14:0 and is not A.

The three main multiple-branched acids (4,8,12-trimethyltridecanoic, pristanic, and phytanic), and the common *Iso* and *Anteiso* fatty acids, may thus be determined by packed column gas–liquid chromatography by multiple analyses on SE-30 and EGSS-Y columns. It may be noted that the 15:0 peak is particularly useful as an internal standard in both hydrogenated materials or isolates of saturated esters since it is of the same magnitude as the branched-chain materials and not obviously coincident with any other material on SE-30 and EGSS-Y under the operating conditions specified.

OPEN-TUBULAR COLUMNS

The study of the saturated materials in marine oils by open-tubular gas–liquid chromatography was initiated with the hydrogenated esters from the triglycerides of cod liver oil (Fig. 2). The analysis on an SE-30 column was not as definitive as anticipated owing to tailing of peaks and the unexpected complexity of the chromatogram in the C_{14}–C_{18} region. A plot of logarithm relative retention time versus carbon chain length (Fig. 3, Table I) gave the usual line for 14:0, 16:0, 18:0, and 20:0. However, the points for the rather obvious 15:0, 17:0, and 19:0 components lay above the line for the other normal fatty acids. This was apparently a further instance of the effect of relative concentration on retention time (Ackman, 1965). The point was investigated with a mixture of 14:0, 16:0, and 18:0 to which small increments of 17:0 were added. As indicated by the ECL values in Table II, the assumption of increased retention time for very small samples was correct, and it may be noted that when the proportion of 17:0 exceeded that of the other components the ECL value for 17:0 was less than 17.00.

It may be assumed that all other minor components in the SE-30 analysis would also be shifted by the relative concentration effect. Since these are all of roughly the same order of magnitude the effect handicaps identifications based on calculation from basic structural considerations (see below). The shortening of retention time was in fact also demonstrated with coinjection of a modest sample of methyl phytanate (Fig. 2).

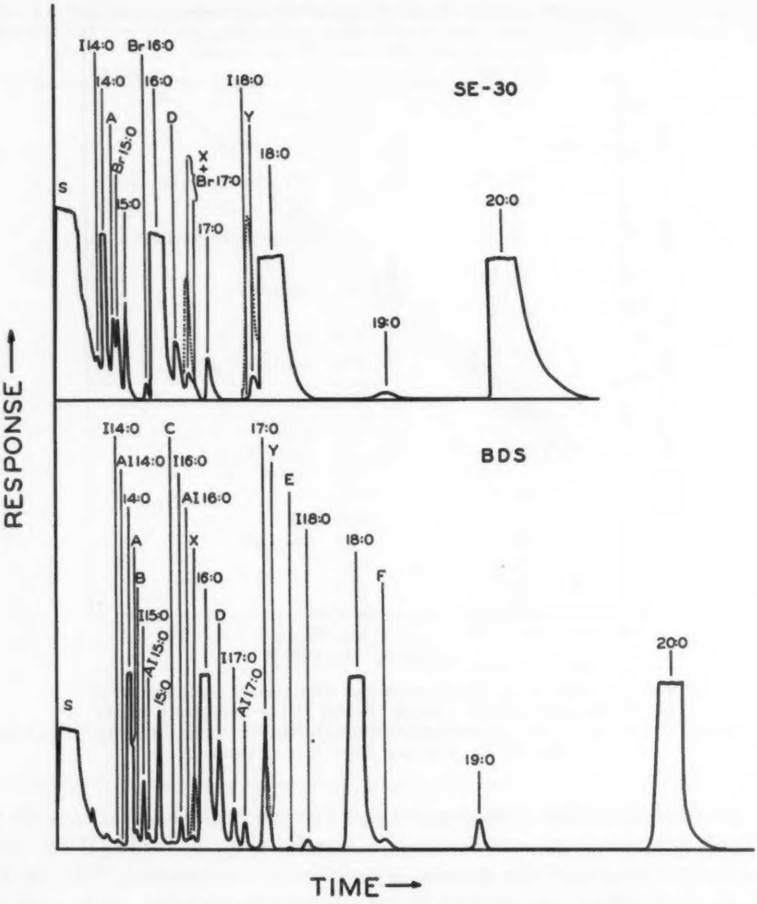

FIG. 2. Partial gas–liquid chromatograms of the hydrogenated methyl esters from cod liver oil triglyceride fatty acids. Open-tubular columns, liquid phases SE-30 and BDS. Experimental conditions and notation given in text. Dotted peaks are from coinjection of sample and authentic methyl pristanate and phytanate.

The SE-30 analysis on the open-tubular column did show resolution from the branched chain C_{15} acids of the peak (A) assumed to be methyl 4,8,12-trimethyltridecanoate. Methyl pristanate could not be selected from the C_{17} branched materials owing to the relative concentration effect, but methyl phytanate could be distinguished from *Iso* 18:0 at the normal concentration encountered in the hydrogenated sample. An additional component (D) of magnitude comparable to the expected branched chain materials was observed. On the log plot this was not quite colinear with A when placed in approximately related positions of whole carbon number.

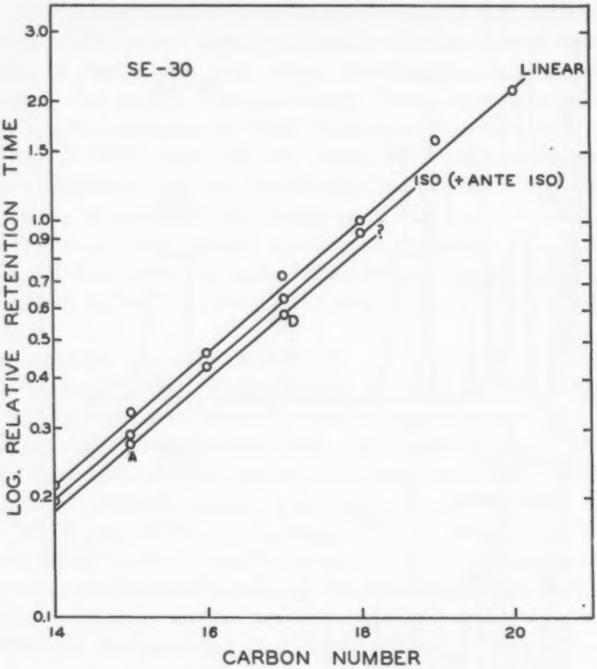

FIG. 3. Plot of log relative retention time against carbon number for SE-30 open-tubular analysis. A and D not necessarily of the carbon numbers shown.

The analysis of the hydrogenated cod liver oil triglyceride sample on the BDS polyester columns was extremely successful (Fig. 2, Table III) in terms of peak symmetry and the general separation of components. The log plot (Fig. 4) showed that the normal linear odd-numbered fatty acids were not subject to a retention time shift due to relative concentration. The respective *Iso* and *Anteiso* acids were colinear in this plot and a further line could be drawn linking a number of minor components (B, C, E, and F) with the somewhat larger unknown component D also recognized on the SE-30 column. The separation of A from 14:0 was incomplete, although on a further analysis on a more efficient (new) BDS column nearly complete baseline separation was obtained. Methyl pristanate was almost coincident with *Anteiso* 16:0 and methyl phytanate appeared in the latter half of the 17:0 peak (Fig. 2) under the particular operating conditions employed with the first column.

The position of methyl 3,7,11-trimethyldodecanoate was well before 14:0 (Table IV) and definitely set it apart from A. There was no significant peak for a component corresponding to 3,7,11-trimethyldodecanoic acid in the cod liver fatty acids in comparison with the amounts of A, pristanate, and

TABLE I. Relative retention times of some shorter-chain esters in a sample of hydrogenated esters from cod liver oil triglyceride fatty acids. Open-tubular column, SE-30 liquid phase. Experimental conditions and notation given in text.

Fatty acids		
Iso	14:0	0.196
	14:0	0.214
4,8,12-Trimethyltridecanoic	(A)	0.272
Iso	15:0	0.287
Anteiso	15:0	
	15:0	0.329
Iso	16:0	0.429
Anteiso	16:0	
	16:0	0.461
	D	0.580
Iso	17:0	0.635
Pristanic	(X)	
Anteiso	17:0	
	17:0	0.727
Iso	18:0	0.928
Phytanic	(Y)	0.942
	18:0	1.00
	19:0	1.59
	20:0	2.13
(Ethyl	16:0)	0.632

TABLE II. Equivalent chain length values for increments of 17:0 added to a mixture of equal amounts of 14:0, 16:0, and 18:0; SE-30 column.

17:0 as per cent of total	ECL value
0.07	17.22
2.0	17.17
5.0	17.12
20	17.01
40	16.90

phytanate. A small peak at the level of one-fiftieth of A was in the right position but associated with several other trace peaks of the same or lesser magnitude. It is possible that losses of materials of such a short chain length could have occurred during manipulation (Ackman and Burgher, 1964b), but not to the extent of more than 20%.

With accurate ECL values available for esters of three multiple-branched fatty acids of known structure it was possible to calculate additive ECL values for the three known structures and for A (Ackman, 1967). As shown in Table

TABLE III. Relative retention times and equivalent chain lengths of some shorter-chain esters in a sample of hydrogenated methyl esters from cod liver oil triglyceride fatty acids, with comparable data from Sano (1966). Open-tubular column, BDS liquid phase. Experimental conditions and notation given in text.

Fatty acid		$r_{18:0}$	Equivalent chain lengths				
			Iso	*Anteiso*	Unknown	Multiple-branched	Sano (1966)[c]
Iso	14:0	0.199	13.55	–	–	–	13.6
	14:0	0.233	–	–	–	–	–
4,8,12-Trimethyltridecanoic	(A)	~0.250[a]	–	–	–	~14.18[a]	14.3(5)
	B	0.263	–	–	14.33	–	14.4
Iso	15:0	0.286	14.54	–	–	–	14.6
Anteiso	15:0	0.305	–	14.73	–	–	–
	15:0	0.337	–	–	–	–	–
	C	0.382	–	–	15.35	–	15.4
Iso	16:0	0.413	15.56	–	–	–	15.6
Anteiso	16:0	0.444	–	15.76	–	–	–
Pristanic	(X)	0.450	–	–	–	15.80	16.2(5)
	16:0	0.485	–	–	–	–	–
	D	0.546	–	–	16.33	–	16.4
Iso	17:0	0.597	16.56	–	–	–	16.6
Anteiso	17:0	0.633	–	16.75	–	–	16.8
	17:0	0.700	–	–	–	–	–
Phytanic	(Y)	~0.715[b]	–	–	–	~17.08[b]	17.5
	E	0.791	–	–	17.35	–	17.4
Iso	18:0	0.852	17.56	–	–	–	17.6
	18:0	1.00	–	–	–	–	–
	F	1.12	–	–	18.33	–	18.4
	19:0	1.44	–	–	–	–	–
	20:0	2.06	–	–	–	–	–
(Ethyl	16:0)	0.550	–	–		16.35	–

[a] Analysis on a superior column gave r 0.231, ECL 14.14.
[b] Analysis on a superior column gave r 0.703, ECL 17.02.
[c] Does not include a series with FCL 0.2.

IV good agreement was obtained for the esters of the three fatty acids of known structure and also for A taken as methyl 4,8,12-trimethyltridecanoate. Subsequently the authentic multiple-branched fatty acid characterized by Sen Gupta and Peters (1966) confirmed this identification.

The effect of temperature on the separation of branched-chain components from normal linear acids was studied by comparative analysis of authentic methyl phytanate and 17:0 at 150 C with the new BDS column. At this temperature phytanate was partially separated from 17:0 and moreover resolved into two components (ECL values approximately 17.05 and 17.08). A similar separation was obtained with authentic pristanate. These components occur as

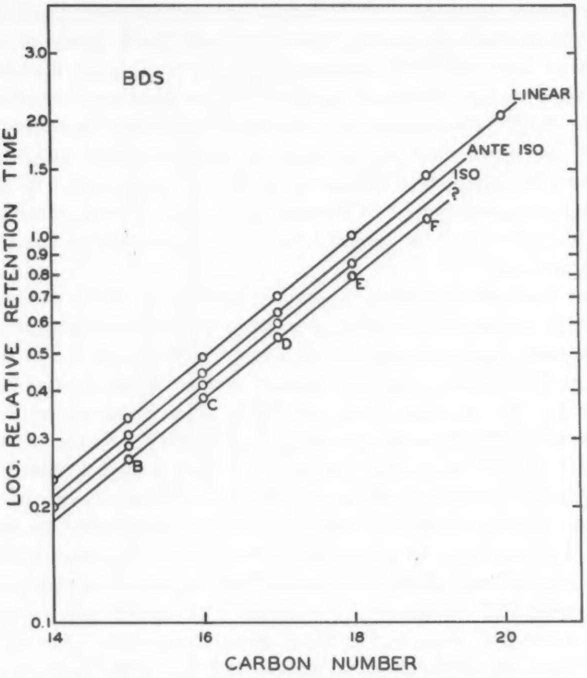

FIG. 4. Plot of log relative retention time against carbon number for BDS open-tubular analysis. B–F not necessarily of the carbon numbers shown.

TABLE IV. Relative retention times and equivalent chain lengths of methyl esters of some multiple-branched fatty acids and normal fatty acids. Open-tubular column, BDS phase. New BDS column (not as for Table III) operated at 150 C and 40 psig helium.

Fatty acid	r18:0	ECL	
		Found	Calculated
3,7,11-Trimethyldodecanoic	0.125	12.87	12.83
14:0	0.198	–	–
4,8,12-Trimethyltridecanoic (A)	0.210	14.14	14.14
2,6,9,12-Tetramethylpentadecanoic (X)	0.413	15.82	15.81
16:0	0.446	–	–
17:0	0.666	–	–
3,7,11,15-Tetramethylhexadecanoic (Y)	0.673	17.02	17.06
18:0	1.00	–	–

two diastereomers (Odham, 1965). 4,8,12-Trimethyltridecanoate showed only one peak. The presence in marine lipids of diasteomeric forms of pristanic and phytanic acids, but not of 4,8,12-trimethyltridecanoic acid, clarifies the origin of these acids. This and biological implications are discussed elsewhere (Ackman and Hansen, 1967). The isothermal operating conditions to achieve this separation would be impractical for analysis of hydrogenated samples due to the excessive retention time, but isolates of saturated acids which do not normally contain significant amounts of 22:0 could be studied. Under conditions for more rapid analysis (170 C) it is unlikely that the separation of the stereoisomers would be significant.

Shifts of multiple-branched materials, relative to other saturates, may be significant with temperature under appropriate conditions (Polgar et al., 1962). Sano (1966) used a polyethylene glycol–adipate column at 215 C, and obtained slightly lower ECL values for the *Iso* and *Anteiso* acids, but markedly larger ECL values for the multiple-branched fatty acids than those in the present study (Table III). On the same polyester, but at 197 C, Sonneveld et al. (1962) found an ECL of 17.1 for methyl phytanate, while Lough (1964a) reported an ECL of 16.9 on EGS (ethylene glycol–succinate) at moderate (probably <175 C) temperatures. Kremer (1965) reported that 20% EGS failed to separate phytanate and 17:0, but that by altering the level of EGS (presumably to a lower level) phytanate fell well after 17:0. Sano (1966) reported two series of unknown compounds, with FCL values of 0.4 and 0.2. The former series is probably the same as the series B–F observed in the present study if the general higher FCL values in his work are applicable. Nicolaides and Ray (1965) report five different series of branched-chain fatty acids in human skin and *vernix caseosa* lipids.

It is apparent that although the BDS open-tubular analysis is highly informative in regard to most of the minor saturated components of marine oils a supplementary analysis on SE-30 is still necessary to enable qualitative and quantitative studies on all of the materials. All of the significant branched-chain and linear odd-numbered fatty acids can be studied by open-tubular gas chromatography without the need for concentration steps. The separation of the *Iso* and *Anteiso* acids of common chain length is particularly useful.

UNKNOWN MATERIALS

The series of components B–F have not been identified. On the SE-30 column, component D is coincident with ethyl 16:0, but on the BDS column the two clearly have different retention times (Tables I and III). The saturates isolated from cod liver oil were no longer available, but those previously isolated from seal oil were examined on the open-tubular columns (Fig 5). The proportions of all components were different from those in the cod liver oil esters, but D could still be recognized. This series of materials (B–F) are therefore unlikely to be artifacts introduced during hydrogenation (Ackman and Burgher, 1964b; Poukka et al., 1962).

In both the current study and that of Sano (1966) esterifications were carried out with boron trifluoride catalysed–methanol solutions. This reagent

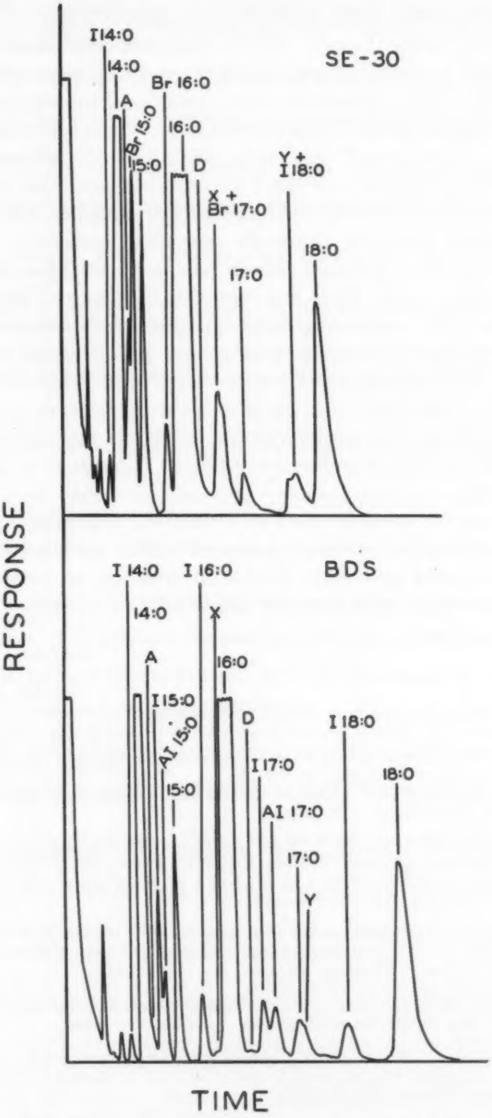

FIG. 5. Partial gas–liquid chromatograms of methyl esters of saturated fatty acids from seal oil. Open-tubular columns, liquid phases SE-30 and BDS. Experimental conditions and notation given in text.

is very powerful and has been shown to give rise to artifacts from reaction with unsaturated fatty acids (Lough, 1964b). Rigorous esterification with sulphuric acid can produce methoxy-stearic acids from trace amounts of hydroxy acids (Hansea, 1966b). The proportions of the unknown materials in the analysis of the hydrogenated cod liver oil triglyceride esters make reaction with unsaturated acids unlikely as a source of this series, since D > E, whereas 16:1 < 18:1 in cod liver oil. The materials are more closely, but not directly, proportional to the saturated acids of cod liver oil (Ackman and Burgher, 1964a). The materials B–F are therefore probably naturally occurring materials although artifacts from unknown parent materials cannot be ruled out. This may also be true of the unknown series with FCL 0.2 reported by Sano (1966). In the present study, using samples containing large amounts of the normal even-numbered fatty acid esters, such materials would only be observed in the regions corresponding to ECL values of 15.2 and 17.2. A few trace components were in fact observed in these and other parts of a chromatogram on a new and highly efficient BDS column, but in the absence of blanks run for comparison of contaminants could not be considered significant (Lindgren et al., 1962; Johnson and Roots, 1964).

Extreme caution must be used in comparing materials by ECL value even when effects such as that of relative concentration are eliminated by the use of polyesters (Nicolaides and Ray, 1965). In addition to isoprene skeletal fatty acids other basic types of structures are possible (Odham, 1965) for multiple-branched fatty acids.

REFERENCES

ACKMAN, R. G. 1965. Effect of relative concentration on retention times of minor components of mixtures. J. Gas Chromatog., **3**: 15–17.

1967. Calculation of ECL values in the gas–liquid chromatography of multiple-branched fatty acids. J. Chromatog. (In press.)

ACKMAN, R. G., AND R. D. BURGHER. 1964a .Cod liver oil: component fatty acids as determined by gas–liquid chromatography. J. Fish. Res. Bd. Canada, **21**(2): 319–326.

1964b. Employment of ethanol as a solvent in small scale catalytic hydrogenations of methyl esters J. Lipid. Res., **5**: 130–132.

ACKMAN, R. G., R. D. BURGHER, AND P. M. JANGAARD. 1963. Systematic identification of fatty acids in the gas–liquid chromatography of fatty acid methyl esters: a preliminary survey of seal oil. Canadian J. Biochem. Physiol., **41**: 1627–1641.

ACKMAN, R. G., AND R. P. HANSEN. 1967. Separation of diastereomers of phytanic and pristanic acids by gas–liquid chromatography. Lipids. (In press.)

ACKMAN, R. G., AND J. C. SIPOS. 1965. Isolation of the saturated fatty acids of marine lipids with particular reference to normal odd-numbered fatty acids and branched-chain fatty acids. Comp. Biochem. Physiol., **15**: 445–456.

AVIGAN, J. 1966a. The presence of phytanic acid in normal human and animal plasma. Biochim. Biophys. Acta, **116**: 391–394.

1966b. Pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) and phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) content of human and animal tissues. Ibid., **125**: 607–610.

BARBER, R. T. 1966. Interaction of bubbles and bacteria in the formation of organic aggregates in sea water. Nature, **211**: 257–258.

Bendoraitis, J. G., B. L. Brown, and L. S. Hepner. 1962. Isoprenoid hydrocarbons in petroleum. Isolation of 2,6,10,14-tetramethylpentadecane by high temperature gas–liquid chromatography. Anal. Chem., **34**: 49–53.

Blumer, M., and D. W. Thomas. 1965a. Phytadienes in zooplankton. Science, **147**: 1148–1149.
1965b. "Zamene" isomeric C$_{19}$ monoolefins from marine zooplankton, fishes and mammals. Ibid., **148**: 370–371.

Blumer, M., M. M. Mullin, and D. W. Thomas. 1963. Pristane in zooplankton. Ibid., **140**: 974 only.
1964. Pristane in the marine environment. Helgolaender. Wiss. Meeresuntersuch., **10**: 187–201.

Cason, J., and D. W. Graham. 1965. Isolation of isoprenoid acids from a California petroleum. Tetrahedron, **21**: 471–483.

Christensen, P. K., and N. A. Sörensen. 1951. Studies related to pristane. V. The constitution of zamene. Acta Chem. Scand., **5**: 751–756.

Christophe, J., and G. Popják. 1961. Studies on the biosynthesis of cholesterol: XIV. The origin of prenoic acids from allyl pyrophosphates in liver enzyme systems. J. Lipid Res., **2**: 244–257.

Eglinton, G., A. G. Douglas, J. R. Maxwell, J. N. Ramsay, and S. Ställberg-Stenhagen. 1966. Occurrence of isoprenoid fatty acids in the Green River shale. Science, **153**: 1133–1135.

Grimmer, G., and J. Jacob. 1965. Occurrence of methyl-branched fatty acids in human blood. Biochem. Z., **341**: 315–324.

Hallgren, B., and S. Larsson. 1963. Separation of pristane from herring. Acta Chem. Scand., **17**: 543–545.

Hansen, R. P. 1964. 2,6,10,14-Tetramethylpentadecanoic acid, a constituent of butterfat. Nature, **201**: 192 only.
1965a. Occurrence of 2,6,10,14-tetramethylpentadecanoic acid in sheep fat. Chem. Ind. London. p. 1258–1259.
1965b. 3,7,11,15-Tetramethylhexadecanoic acid: its occurrence in sheep fat. New Zealand J. Sci., **8**: 158–160.
1965c. 3,7,11,15-Tetramethylhexadecanoic acid: its occurrence in the tissues of humans afflicted with Refsum's syndrome. Biochim. Biophys. Acta, **106**: 304–310.
1966a. Phytanic acid and other branched-chain fatty acid constituents of bovine rumen bacteria. J. Dairy Res., **33**: 333–342.
1966b. Isomeric methoxy-stearic acid artifacts from sheep perinephric fat. Chem. Ind. London. p. 288.

Hansen, R. P., and J. D. Morrison. 1964. The isolation and identification of 2,6,10,14-tetramethylpentadecanoic acid from butterfat. Biochem. J., **93**: 225–228.

Jeffrey. L. M. 1966. Lipids in sea water. J. Am. Oil Chemists' Soc., **43**: 211–214.

Johnson, P. V., and B. I. Roots. 1964. A source of contamination in the ultramicro analysis of methyl esters of fatty acids by gas–liquid chromatography. J. Lipid Res., **5**: 477–478.

Kates, M., L. S. Yengoyan, and P. S. Sastry. 1965. A diether analog of phosphatidyl glycerophosphate in *Halobacterium cutirubrum*. Biochim. Biophys. Acta, **98**: 252–268.

Kremer, G. J. 1965. On the occurrence of 3,7,11,15-tetramethylhexadecanoic acid in lipids of normal sera. Klin. Wochschr., **43**: 517–518.

Lambertsen, G., and R. T. Holman. 1963. Partial characterization of the hydrocarbons of herring oil. Acta Chem. Scand., **17**: 281–282.

Leo, R. F., and P. L. Parker. 1966. Branched-chain fatty acids in sediments. Science, **152**: 649–650.

Lindgren, F. T., A. V. Nichols, N. K. Freeman, and R. D. Wills. 1962. Potential contamination in the analysis of methyl esters of fatty acids by gas–liquid chromatography. J. Lipid Res., **3**: 390–391.

LOUGH, A. K. 1963. Isolation of 3,7,11,15-tetramethylhexadecanoic acid from ox plasma. Biochem. J., **86**: 14 p.

1964a. Blood lipids. 4. The isolation of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) from ox-plasma lipids. Ibid., **91**: 584–588.

1964b. The production of methoxy-substituted fatty acids as artifacts during the esterification of unsaturated fatty acids with methanol containing boron trifluoride. Ibid., **90**: 4c–5c.

MIWA, T. K., K. L. MIKOLAJCZAK, F. R. EARLE, AND I. A. WOLFF. 1960. Gas chromatographic characterization of fatty acids. Ibid., **32**: 1739–1742.

MORRISON, W. R., AND L. M. SMITH. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res., **5**: 600–608.

NICOLAIDES, N., AND T. RAY. 1965. Skin lipids. III. Fatty acid chains in skin lipids. The use of *Vernix Caseosa* to differentiate between endogenous and exogenous components in human skin surface lipid. J. Am. Oil Chemists' Soc., **42**: 702–707.

ODHAM, G. 1965. Studies on feather waxes of birds. III. The chemical composition of the wax in the free-flowing secretion from the preen gland of the mute swan (*Cygnus olor*). Arkiv Kemi, Band 23, Häfte, **5**: 431–451.

POLGAR, A. G., J. J. HOLST, AND S. GROENNINGS. 1962. Determination of alkanes and cycloalkanes through C_8 and alkenes through C_7 by capillary gas chromatography. Anal. Chem., **34**: 1226–1234.

POUKKA, R., L. VASENIUS, AND O. TURPEINEN. 1962. Catalytic hydrogenation of fatty acid methyl esters for gas–liquid chromatography. J. Lipid Res., **3**: 128–129.

SANO, Y. 1966. Studies on the Antarctic whale oils by gas–liquid chromatography using a hydrogen flame ionization detector. IV. Analysis of the branched chain fatty acids from blue whale bone oil. Yukagaku, **15**: 140–147.

SEN GUPTA, A. K., AND H. PETERS. 1966. Isolation and structure determination of the polybranched fatty acids from fish oils. Fette. Seifen. Anstrichmittel, **68**: 349–360.

SONNEVELD, W., P. HAVERKAMP BEGEMANN, G. J. VAN BEERS, R. KEUNING, AND J. C. M. SCHOGT. 1962. 3,7,11,15-Tetramethylhexadecanoic acid, a constituent of butterfat. J. Lipid Res., **3**: 351–355.

SÖRENSEN, N. A., AND J. MEHLUM. 1948. Studies related to pristane. I. The unsaponifiable matter of the liver oil of the basking shark. Acta Chem. Scand., **2**: 140–146.

SÖRENSEN, J. S., AND N. A. SÖRENSEN. 1949. Studies related to pristane. III. The identity of *Nor*phytane and Pristane. Ibid., **3**: 939–945.

SUTCLIFFE, W. H., JR., E. R. BAYLOR, AND D. W. MENZEL. 1963. Sea surface chemistry and Langmuir circulation. Deep-Sea Res., **10**: 233–243.

VANDENHEUVEL, F. A. 1952. Precision semimicro hydrogenation apparatus. Anal. Chem., **24**: 847–851.

WAKABAYASHI, K., AND N. SHIMAZONO. 1963. Studies on the ω-oxidation of fatty acids *in vitro*. I. Overall reaction and intermediates. Biochim. Biophys. Acta, **70**: 132–142.

Postmortem Physicochemical Changes in Unfrozen Newfoundland Trap-Caught Cod[1,2]

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ABSTRACT

Cod (*Gadus morhua*) caught in traps along the shores of Newfoundland are shown to be high in muscle glycogen, reaching a peak in late July and, unfrozen, reach a lower *p*H post-mortem than do cod caught in other Newfoundland fishing operations. Seawater temperature, muscle glycogen at death, and free drip developed in the fillets were minimal in June and maximal in late July. Fillets of these latter fish, after freezing during rigor, were of significantly poorer texture that those prepared earlier and later in the season.

Fish which were not iced as soon as caught entered rigor mortis within 1 or 2 hr and although iced in the interim, were out of rigor in 24–36 hr. Fish iced at the trap were in full rigor 8–15 hr later; although rigor did not disappear completely for 70–90 hr, the fish could be filleted, by hand, in 24 hr.

Generally, there were small weight losses in whole eviscerated fish not iced until several hours after capture but this weight was regained completely after 34 hr.

Whole, eviscerated fish iced at capture developed less drip in the fillets than fish permitted to warm before icing. In fillets of fish in all stages of rigor there was a consistent trend for drip to increase with decreasing *p*H at filleting. The *p*H levels in turn were dependent on season of catch and on glycolysis after killing. In late midseason (August 2) catches, muscle glycogen appeared to be directly responsible for *p*H drop whereas in fish captured earlier, feed, physiological condition, and seawater temperature appeared to be the principal catalysts of *p*H change. Fillets prepared during rigor (August catches) showed no further drop in *p*H from that of the parent, iced material and developed 2–3% free drip in 24 hr; those processed before or after rigor mortis showed a further drop in *p*H from that occurring in the whole fish, and drip developed was much greater than in the samples filleted during rigor. Because considerable variablilty was noted in the rate of rigor development in any one catch, the particular state of rigor at filleting was found to correlate better with the amount of free drip in the fillets than was postmortem age. The development and resolution of rigor in fillets was accompanied by considerable reduction in the solubility of the proteins (in 0.6 M NaCl solution), significantly more than in whole fish.

If, for production reasons, trap fish must proceed into rigor before freezing, they should be chilled and stored in the whole, eviscerated state rather than as fillets.

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[2] This is the twelfth paper on quality of fish. Earlier unnumbered publications in this series are reported by W. A. MacCallum, E. J. Laishley, W. J. Dyer, and D. R. Idler. 1966. Taste panel assessment of cod fillets after single and double freezing. J. Fish. Res. Bd. Canada, **23**(7): 1063–1081.

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INTRODUCTION

About 70 million lb (ungutted weight) of Newfoundland cod taken in the trap fishery (May–August) are frozen annually (A. T. Pinhorn, personal communication). Thus the quality of the frozen, stored pack (see MacCallum et al., 1966) and the prior handling of the material from the traps to the freezers, which may have a bearing on production problems and on the quality of the frozen, stored product, are of great importance to industry. This communication discusses the physical and physicochemical features associated with such handling.

These cod have already spawned (in May — Templeman and Fleming, 1956, 1962) upon arrival at the fishermen's traps ranged along the coast. While near the shore they are exposed to increasing water temperature until early August (MacCallum et al., 1966) and until late July consume increasing quantities of capelin (*Mallotus villosus*) after which the feed intake decreases. In the St. John's area, where these studies were conducted, the fish are relatively small, the major portion being in the 4–6 year age-group and of gutted weight 1.2–4.5 lb (40–65 cm in length — A. M. Fleming, personal communication).

For the most part the time between capture and plate freezing in packages or blocks of finished fillets varies from a few hours to 1 or 2 days. On landing, the fish that are not to be filleted and frozen on the day of capture are usually iced or stored in refrigerated sea water.

Recently, Jones (1965a) showed that toughness in cod fillets, frozen prerigor, increases with increased lactic acid concentration resulting from higher muscle glycogen reserves in the fish at killing. Tomlinson et al. (1965) found that chalkiness in halibut was dependent on the postmortem development of low muscle *p*H and was accompanied by a marked decrease in solubility of the proteins in both high and low ionic strength salt solutions. MacCallum et al. (1966) associated the texture of frozen stored trap cod with season of catch and of Grand Bank cod with the *p*H of the fillets at freezing. Fraser et al. (1965) showed samples from one lot of trap fish to be relatively high in muscle glycogen whereas J. T. Lauder and the present authors (unpublished results) showed both muscle glycogen and feed in trap fish to peak at about the same time (late July). Tomlinson et al. (1966) working with iced halibut found free drip to increase continuously with decreasing *p*H. Notwithstanding, there is a lack of specific data showing the effect of *p*H (before freezing) on processing procedures, yields, and on quality after frozen storage. Few facts are available on correlation among glycogen content, *p*H, and free drip in unfrozen fish muscle and, in particular, trap-caught cod.

Publications on free drip (formed without the application of force) from unfrozen fish tissue and on weight losses resulting from vessel or plant handling treat primarily with cod stored for long periods (Ellison, 1934; Dyer and Dyer, 1947; Cutting, 1951). However, Banks (1955) obtained 30% more *expressible* juice from unfrozen fillets cut from gutted cod previously iced for 40 hr than from those iced 18 hr (rigor not resolved) and Partmann (1960) found an increasing loss in the water-binding capacity of carp muscle after the fish had been stored at 10 C for 1 day. Fluid retention by fish muscle is known to be extremely variable and is no doubt influenced by physiological conditions and such factors as feed and water temperature. With trap fish, environmental factors may be extreme (MacCallum et al., 1966), which would suggest that these fish might be particularly susceptible to postmortem exudation of drip.

PRELIMINARY EXPERIMENTS

Influence of Environmental, Biological, and Physicochemical Conditions Obtaining at Capture and After Storage

In 1960 data were obtained on the temperatures of trap fish at time of capture and on the feed in the gut, and fish and fillets were assessed organoleptically and the *p*H of the surface of the fillet (from 2 to 25 hr post-mortem) was measured. One-half of a live catch sampled on June 28 as eviscerated on the trap boat, the other half not. Immediately thereafter, each of these two lots was portioned in four and the fish iced or not iced and handled normally or maltreated in eight aluminum boxes each of approximately 200 lb capacity. To maltreat the cod a board was laid across the top layer of fish and a 35-lb weight dropped a distance of 1 ft onto the board on 10 successive occasions. The fish were stored with or without ice at room temperature (27 C) for a period of about 24 hr. During the first 3 hr after capture, the top fish without ice were exposed to a hot sun. Another sampling was made next day when ungutted fish were boxed without ice for 2¼ hr after brailing from the traps and were then iced for an additional period of 17 hr under the same storage conditions as the previous day's samples.

The gut of each cod examined contained from two to five capelin in preliminary stages of digestion. The fish were of the following sizes and ages:

	Length (*cm*)	Gutted weight gills (*lb*)	Year-class	Age	Percentage of catch
June 28 catch	43–50	1.4–2.2	1956	4	40
	51–54	2.3–2.6	1955	5	15
	55–59	2.8–3.5	1954	6	30
	–	–	Older year-classes		15
June 29 catch	51–54	2.3–2.6	1955	5	14
	55–59	2.8–3.5	1954	6	42
	61–64	3.7–4.3	1953	7	10
	65–68	4.5–5.0	1952	8	17
	–	–	Older year-classes		17

The above data show clearly the mixing of year-classes in any one trap load and the considerable difference in fish size which may be encountered from day to day.

The June 28 catch, at a temperature of 6.6 C when boated and iced, ranged in temperature from 0 C to 2 C after 3 hr. The fish were not then in rigor mortis. The temperature of the individuals not in ice was 21.2–25.8 C at this time; some of the fish were in rigor. Later work has shown that a temperature range of 9–13 C about 4 hr post-mortem and a maximum of 17 C 5 hr post-mortem for cod not iced are more typical of conditions in the St. John's area. After 24 hr the *p*H of the June 28 iced fish was 6.04 ± 0.03 (50 samples) as measured using a Beckman 110-v, model H2 *p*H meter. The fish were still partly in rigor. The whole fish and the fillets, prepared at 24 hr, were firm. The samples without ice and the fillets removed from them were soft, the flesh gaping, the *p*H 6.29 ± 0.04 (20 samples).

The June 29 catch, in rigor after $2\frac{1}{4}$ hours, had warmed to 15.4–19.5 C. After icing for an additional 17 hr the temperature range was 0.5–8.0 C. The *p*H of fillets excised at the former time was 6.12 ± 0.06 ($n = 22$) and at the latter, 6.12 ± 0.03 ($n = 40$), the values being independent of year-class.

Softening to the point where the muscle appeared to be partly cooked was much in evidence under the skin of the fish that were exposed to the sun. After 24-hr storage there was breakdown in the gut wall in some of the ungutted fish held both in and out of ice. Thus, trap-caught cod which cannot be processed on the day of capture should be gutted before storing. The degree of softening of the flesh, not located next to the gut wall, appeared to be the same in both gutted and ungutted samples held at elevated temperatures; the softening appeared to be independent of harsh physical treatment but dependent on temperature.

DEVELOPMENT OF RIGOR MORTIS (FIG. 1)

Figure 1 illustrates the effect of two treatments on the development of rigor in trap fish. Figure 1A depicts fish for the main part captured shortly after the start of the feeding period in 1960 and in 1961, then killed and stored initially without ice. The fish proceeded into rigor within the first hour or two after capture and, depending upon intrinsic characteristics and handling temperatures (including later icing as practiced in the fish plants), passed completely through rigor in 24–36 hr. Some of the fish became quite stiff initially, others were of a rubbery consistency in contrast with uniform rigidity in fish killed and iced at the traps (see below).

About half of the fish captured at the height of the feeding period in 1961 (and at near maximum water temperatures), killed and iced at capture (Fig. 1B) were rigid after about 8–10 hr; all were in rigor at about 15 hr. Although rigor did not disappear completely for 70–90 hr, the fish could be filleted, by hand, in 24 hr.

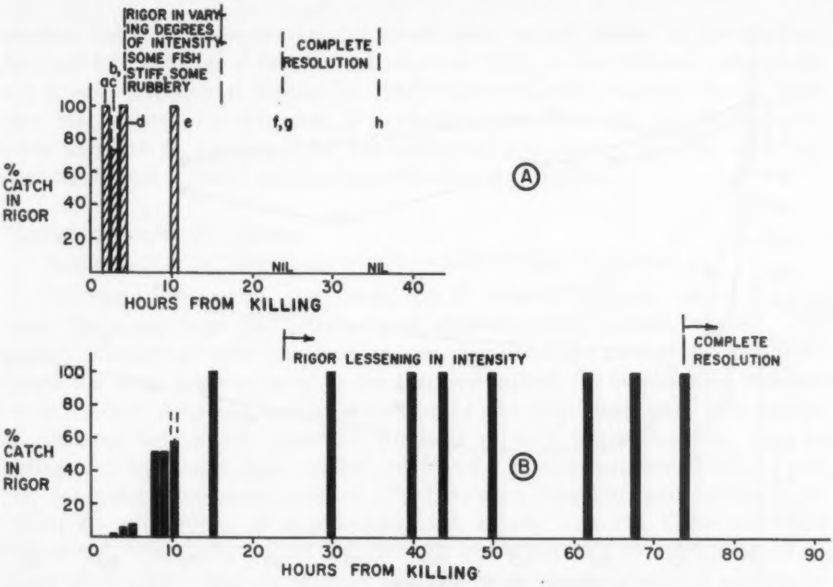

FIG. 1. Bar chart showing the onset and duration of rigor in relation to the handling of Newfoundland trap-caught cod. A, late June and early July fish (exception sample (*e*)), stored without ice at killing and, where specified, iced later: (*a*) ungutted samples, not iced at killing on June 29, 1960, temperature 2¼ hr later (at icing), 15–19 C; (*b*) gutted samples, not iced at killing on June 28, 1960, temperature 3 hr later (at icing), 21–26 C; (*c*) treatment as in (*b*), temperature 3 hr post-mortem (at icing), 9–13 C (NOTE: The cross-hatched area (bars *b*, *c*) depicts the approximate value (% catch in rigor) in the two instances, the broken lines the possibility that the value could be considerably higher); (*d*) treatment as in (*b*), temperature 2½ hr post-mortem (at icing), 7 C; (*e*) treatment as in (*b*) but captured on July 31, 1962, temperature 10 hr post-mortem (at icing), 15 C; (*f*) treatment as in (*a*), but iced for an additional 17 hr; (*g*) treatment as in (*b*), but iced for an additional 21 hr; (*h*) treatment as in (*b*), but captured about July 10, 1961, temperature 2 hr later (at icing), 18 C; iced for an additional 34 hr. B, fish gutted and iced at killing, July 20–22, 1961 (NOTE: For explanation of broken lines, see Note accompanying (*b*) and (*c*) above).

POSTMORTEM DROP IN *p*H (FIG. 2)

These typical curves show low *p*H values post-mortem, particularly in the fish captured on July 20–22, 1961 and on July 2, 1963 and held for 3 days. The decrease in the *p*H of these lightly exercised trap fish after killing, as in those examined in 1960, was very significant compared to that reported by MacPherson (1932) for tank-held (lightly exercised) haddock (*p*H drop from 6.99 to 6.47), and by Partmann (1964) who reported *p*H values between 6.2 and 6.6 for fish (of many species) in full rigor and cites a low value of 5.94 for but a single cod in a lot of over 200 trawled fish. Parallel with the development of firm rigor in the trap-caught cod killed and iced at capture, the *p*H was seen to drop rapidly to about 6.0 or 6.1 and to remain at this low level for almost 2 days (curve *a*). A still faster drop was experienced in the samples iced on July 11, 1963 after storage without ice for 1 hr (curve *b*). The samples handled on July 2, 1963 experienced a significant drop in

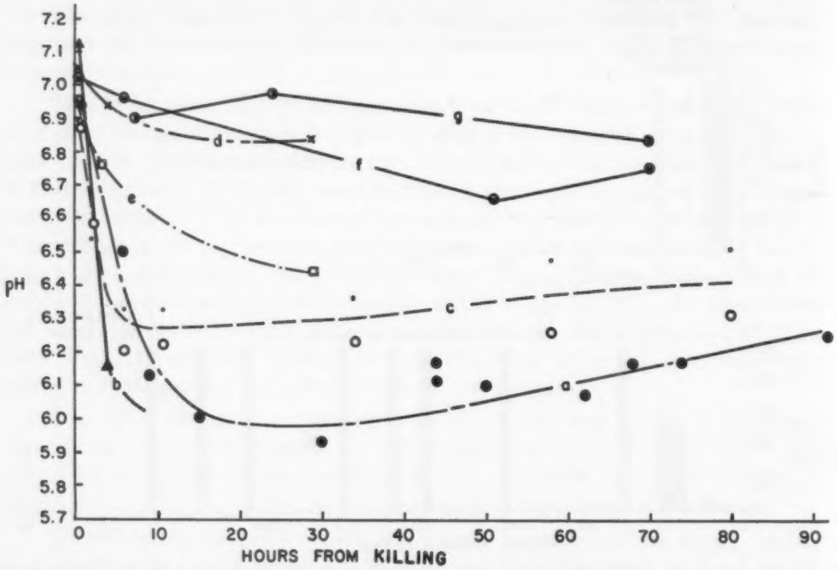

FIG. 2. The *p*H-time curves for iced Newfoundland cod. LEGEND: (*a*) — — —, an average of points for fish taken in the July 20–22 period, 1961. Each point is the average of two readings (●) in each of the two fish bled, gutted, and iced at killing at the trap; (*b*) ▲—▲, average of 10 fish killed at the trap on July 11, 1963 held ungutted for 1 hr at 18 C ambient, then gutted and iced; (*c*) – – – –, average of points for three fish of one catch (July 2, 1963) bled, gutted, and iced at trap (●) and for another three handled ungutted in ice (○); (*d*) — – – – —, average of points (×) each of which is the average of 40 fish bled, gutted, and iced at the trap (June 21, 1965); (*e*) —·—, average of points (□) each of which is the average of 12 fish bled, gutted, and iced at the trap (August 2, 1965); (*f*) ◒—◒, each point is the average of five cod taken alive after a ½-hr tow (otter trawl) 25 miles southeast of St. John's on February 7, 1962. The fish were bled, gutted, and iced immediately; (*g*) ◑—◑, each point is the average for 10 shore cod taken alive on baited trawl (lines in water for 2 days) in February 1962, and immediately bled, gutted, and iced (example of 'alkaline' rigor).

*p*H post-mortem but the level (curve *c*) is a full 0.2 *p*H units above that for the individuals exposed to warm water and continuous feeding for another 9 days (curve *b*). The drop in *p*H post-mortem was still smaller in early season (curve *d*) and in late season (curve *e*) samples examined in 1965. A comparison of the above with curves *f* and *g* for cod trawled and line-caught respectively in February shows that for the most part trap fish experience a much greater drop in *p*H post-mortem than do cod obtained in other Newfoundland operations. Low postmortem *p*H, characteristic of high muscle glycogen in the live animal, thus appeared to be a unique characteristic of the trap cod population and indicated a need for further direct and related studies.

THE MAJOR INVESTIGATIONS

OUTLINE

In 1964 and 1965 data were obtained on the temperature of the sea water and the fish at time of capture, feed in the gut, the effect of a range of post-mortem holding temperatures and conditions, weight losses in gutted fish, *p*H, and free drip (as a function of state of rigor) in the fish and fish fillets, and muscle glycogen at killing. In 1965 frozen samples prepared during rigor were taste tested for purposes of correlating textures with initial glycogen levels and *p*H at freezing. The variability of *p*H, rigor, free drip, and salt extractable (0.6 M NaCl solution) protein was also studied.

PROCEDURES AND METHODS

SAMPLES FOR DETERMINING WEIGHT LOSSES, FREE DRIP, AND *p*H

On several occasions, cod, 50–62 cm in length, obtained about 3 miles along the coast from St. John's, were stunned, bled, gutted, washed, and tagged. Records of size, gut content, sex, temperature, and *p*H of the individual fish were made as soon as the fish were killed. All fish handled without ice in 10-inch deep aluminum boxes aboard the trap boat were iced ashore in insulated bins which permitted drainage of melt water from the fish. In timing, the icing operation, carried out 5 and 9 hr after capture(Tables I and II), simulated commercial practice. The bins were stored at room temperature (21–24 C) with 10 to 15 experimental fish stowed in each. These cod were laid in one layer on a bed of ice and the whole covered to a total depth of about 2½ ft with a mixture of other fish and ice to simulate normal loading on the experimental samples. Stowing was completed with a 6-inch layer of ice. For comparison, fish were also iced in aluminum boxes on the trap boat and were re-iced ashore in the bins.

The fish were weighed individually ashore shortly after capture (1–2 hr after killing, 1964 samples; 5 hr after killing, 1965 samples) and periodically after further storage in ice. A Berkel 10 Kg platform scale was used. At each weighing, observations on rigor, *p*H, and temperature were made. On these occasions, clinging ice was removed by dipping the fish in ice water; the samples drained for 2 min on a wire screen and, after weighing, immediately re-iced.

The *p*H of whole, eviscerated fish and fillets was obtained using a combination glass/calomel electrode, in the former by using a fresh incision below the first dorsal fin and in the latter by pushing the tip of the electrode into the cut surface at the thick portion of the fillet about one-third the distance from the head to the tail.

Skinned fillets were prepared from a proportion of the eviscerated fish. The fillets were handled dry (without water conveyance). Free drip was collected in a vessel from the fillets laid on a mesh screen which, in turn, was supported above the fluid, the whole being enclosed in airtight polyethylene to prevent evaporation, and stored at 4 C. The fillets were weighed at the start and after various periods of storage, and the results used in calculating percentage free drip.

The filleting referred to above was carried out on fish of various treatments (Table II). Some cod on which ice was not used were filleted at sea shortly after capture or ashore after 5 or 9 hr postmortem storage. Other fish, kept without ice for 5 or 9 hr, were then iced and stored for a further

TABLE I. Weight changes and *p*H developed in whole gutted trap-caught cod captured during the 1964 and 1965 fishing seasons and stored as indicated.

				Storage of whole, eviscerated fish									
				Time (*hours*)			Temperature (*C*)		*p*H after storage (*hours from capture*)				
Item No.	Date of catch	Sex	Feed	Not iced	Iced	Total	At killing	At icing	5–9	24–29	34	48–53	72–74
Delayed icing													
1	June 19	Female	Light	9	63	72	6	16	6.83	6.75	–	–	6.82
2	June 25	Male	Light	9	44	53	3	5	6.75	–	6.56	6.68	–
3	July 28	Female	Heavy	9	39	48	4	20	6.35	6.45	–	6.35	–
4	Aug. 10	Male	Light	5	24	29	7	17	6.34	6.26	–	–	–
5	Aug. 10	Male	Light	5	48	53	7	17	6.50	–	–	6.63	–
Iced immediately													
6	June 15	Male	Light	0	74	74	6	6	–	6.86	–	6.81	6.89
7	June 25	Female	Light	0	53	53	3	3	–	–	6.61	6.70	–
8	July 28	Female	Heavy	0	48	48	4	4	–	6.47	–	6.50	–
9	Aug. 23	Male	Light	0	29	29	12	12	6.65	6.70	–	–	–
10	Aug. 23	Female	Light	0	29	29	12	12	6.83	6.80	–	–	–
11	Aug. 23	Male	Light	0	53	53	12	12	6.68	6.71	–	6.66	–
12	Aug. 23	Female	Light	0	53	53	12	12	6.69	6.72	–	6.62	–

(*Continued*)

Table I. Weight changes and *p*H developed in whole gutted trap-caught cod captured during the 1964 and 1965 fishing seasons and stored as indicated. — (*Continued*)

	Per cent weight change after storage (*hours from capture*)									
	Average loss (–) or gain (+) for all samples					Maximum loss (–) or gain (+) in individual fish				
Item No.	9	24–29	34	48–53	72–74	9	24–29	34	48–53	72–74
1	+0.5	–0.3	–	–	+1.3	–0.9	–6.0, +0.7	–	–	–0.2, +5.2
2	+0.03	–	–0.4	+0.9	–	–0.6,+1.0	–	–1.0, +0.1	+1.3	–
3	+0.5	–0.8	–	–0.3	–	–0.7	–2.2	–	–2.3, +1.0	–
4	–	+0.6	–	–	–	–	+1.3	–	–	–
5	–	–	–	+0.9	–	–	–	–	+1.6	–
6	–	+0.5	–	+0.7	+1.7	–	+1.2	–	–2.7, +1.8	+3.2
7	–	–	–0.2	+0.8	–	–	–	–2.5, +2.0	+1.8	–
8	–	+0.2	–	+0.4	–	–	–0.2, +0.5	–	+0.9	–
9	–	+0.2	–	–	–	–	–0.5, +0.5	–	–	–
10	–	+0.6	–	–	–	–	+1.2	–	–	–
11	–	+0.1	–	+0.5	–	–	–0.4, +0.7	–	–0.2, +1.0	–
12	–	+0.8	–	+0.3	–	–	+2.5	–	–0.8, +2.5	–

(*Continued*)

TABLE I. Weight changes and *p*H developed in whole gutted trap-caught cod captured during the 1964 and 1965 fishing seasons and stored as indicated. — (*Concluded*)

	Number of samples showing change after (*hours*)														
	9			24–29			34			48–53			72–74		
Item No.	Gain	Loss	No change	Gain	Loss	No change	Gain	Loss	No change	Gain	Loss	No change	Gain	Loss	No change
1[a]	–	19	1	10	4	1	–	–	–	–	–	–	7	2	–
2	3	5	2	–	3	2	1	3	1	5	–	–	–	–	–
3	–	10	–	–	10	–	–	–	–	4	5	–	–	–	–
4	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–
6	–	–	–	7	–	3	–	–	–	9	1	–	10	–	–
7	–	–	–	–	–	–	3	5	1	8	–	1	–	–	–
8	–	–	–	7	1	2	–	–	–	8	–	2	–	–	–
9	–	–	–	4	1	–	–	–	–	–	–	–	–	–	–
10	–	–	–	4	–	1	–	–	–	–	–	–	–	–	–
11	–	–	–	3	2	–	–	–	–	4	1	–	–	–	–
12	–	–	–	4	–	1	–	–	–	2	3	–	–	–	–

[a] In this section of the table, reductions in the total number of fish reported after a particular storage period in relation to those for a preceding period are due to the use of some fish as fillet samples (the latter reported in Table II).

TABLE II. Free drip and *p*H developed in fillets of trap-caught cod captured during the 1964 and 1965 fishing seasons and handled with and without ice for various periods.

				Storage of whole, eviscerated fish							
				Time (*hours*)			Temperature (*C*)			Rigor	
Item No.	Date of catch	Sex	Feed (capelin)	Not iced	Iced	Total	At killing	Before icing	At filleting	At filleting	When resolved (*AF* = *after filleting*)
1	June 15	Male	Light	0	4	4	6	6	3	Pre	48 hr *AF*
2	" 19	Female	"	9	0	9	6	–	16	Incipient to full	15 hr *AF*
3	" 19	Female	"	9	16	25	6	16	1	Resolved	–
4	" 25	Male	"	9	0	9	3	–	5	Incipient	15 hr *AF*
5	" 25	Female	"	0	0	0	4	–	4	Pre	Not known
6	July 28	"	Heavy	9	25	34	4	20	1	Resolved	–
7	July 28	"	Heavy	0	34	34	4	4	1	Resolved	–
8	Aug. 10	Male	Light to empty	5	0	5	7	–	17	Pre	24–48 hr *AF*
9	" 10	"	" " "	5	24	29	7	17	0	Full	5 hr *AF*
10	" 10	"	" " "	5	24	29	7	17	0	Partly resolved	5 hr *AF*
11	" 10	"	" " "	5	24	29	7	17	0	Resolved	–
12	" 10	"	" " "	5	48	53	7	17	0	Resolved	–
13	" 23	"	" " "	0	5	5	12	12	1	Pre	48 hr *AF*
14	" 23	Female	" " "	0	5	5	12	12	1	Pre	48 hr *AF*
15	" 23	Male	" " "	0	29	29	12	12	0	Full	5 hr *AF*
16	" 23	Female	" " "	0	29	29	12	12	0	Full	5 hr *AF*
17	" 23	Male	" " "	0	53	53	12	12	0	Resolved	–
18	" 23	Female	" " "	0	53	53	12	12	0	Resolved	–

(*Continued*)

TABLE II. Free drip and *p*H developed in fillets of trap-caught cod captured during the 1964 and 1965 fishing seasons and handled with and without ice for various periods. — (*Concluded*)

Item No.	Corresponding item No. in Table I	*p*H at filleting		Free drip[a] from fillets after (*hours*)		
		Whole fish	Fillets	5	24	48
1	N/A	6.90	6.90	–	1.5	2.5
2	(1)	6.83	6.79	–	5.0	6.8
3	(1)	6.75	6.66	–	4.9	6.1
4	(2)	6.75	6.75	–	4.3	6.5
5	N/A	–	7.05	–	3.7	5.7
6	(3)	6.40	6.40	–	6.0	8.0
7	(8)	6.40	6.40	–	3.6	6.0
8	N/A	6.50	6.47	2.9	5.6	7.5
9	(4)	6.32	6.28	0.8	2.9	3.7
10	(4)	6.24	6.22	1.9	5.2	6.2
11	(4)	6.10	6.14	6.5	9.1	10.9
12	(5)	6.63	6.56	1.0	3.4	4.8
13	N/A	6.71	6.75	1.2	4.1	7.5
14	N/A	6.78	6.72	1.1	3.6	6.0
15	(9)	6.70	6.71	1.1	2.2	3.3
16	(10)	6.80	6.78	0.8	1.9	3.2
17	(11)	6.66	6.66	1.1	2.7	4.1
18	(12)	6.62	6.52	1.9	3.6	5.3

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[a] Expressed as % weight of skinned fillet.

period before filleting. Fish iced at the traps were filleted after periods of iced storage varying from 4 to 53 hr. The weight, *p*H, state of rigor, and temperature of all whole fish and fillets were recorded at filleting time and after 24 hr and 48 hr fillet storage at 4 C and, in some instances, after shorter and longer periods of storage. The fillets were weighed on a 3 Kg Kilomat platform scale.

SAMPLES FOR GLYCOGEN, EPN, *p*H AND RIGOR DETERMINATIONS AND FOR FREEZING

On three occasions in 1965 (June 21 (78 samples), July 19 (15 samples), and August 2 (24 samples)) fish were brailed live from the trap into a seawater tank aboard the research vessel, the fish undergoing the same struggling as those consigned to commercial processing. Immediately, individual fish were removed from the tank, stunned, bled, and sampled for glycogen. Also, on June 21, 10 fish which appeared to be exercised very little in the trap were handled with special care. Each individual was carefully lifted from the sea, stunned, bled, and sampled for glycogen before the next fish was handled.

For all fish, about 5 g of muscle was excised from the area under the first dorsal fin, care being taken to sample all fish in exactly the same position, and immediately placed in tubes containing 10 g 30% KOH. These tubes were held chilled in ice on the vessel and at 4 C ashore until digestion could be completed. In the laboratory the known weight of muscle in the tubes was digested in a water bath at 100 C for 1 hr. After cooling, the digests were made to 25 ml and two 5-ml aliquots taken. Glycogen was precipitated with ethanol according to Carroll et al. (1956) followed by colour development with the phenol–sulphuric acid reagent of Montgomery (1957).

All the fish brailed commercially and sampled for glycogen were iced at sea for further processing and organoleptic assessment. The fish were filleted, skinned, packed, and frozen between plates during rigor (freezing time 1 hr). About 1 month later the packages were removed from −23 C storage, sliced with a motorized saw, and the unthawed slices baked for 15 min at 268 C.

Still other fish, brailed commercially, were stunned, bled, eviscerated, and iced at the traps on August 17 and August 23, 1965, and were examined for *p*H and state of rigor and sampled for salt soluble protein in the unfrozen fillets. Salt soluble protein values (EPN) were determined using 22.5-g samples, extracting for 2.5 min with 0.6 M NaCl (0.003 M $NaHCO_3$) in a Waring Blendor (500-ml jar) fitted with a baffle plate, at a speed of 7000 rpm. The extracts were centrifuged for 30 min at 2000 rpm (Dyer et al., 1950, 1964). Colorimetric readings were made after the addition of biuret solution following the procedure of Snow (1950).

EPN values and taste panel ratings of texture were made over a period of 8 days on whole, eviscerated fish and fillets of the August 17 catch. Half of the catch was filleted before rigor and stored (skins-on) in polyethylene bags in ice (so that the fillets remained in their own drip), the remainder held whole in ice. The stored fillets were skinned, sampled for EPN, and taste tested (unfrozen fillets baked at 260 C) 5, 29, 53, and 200 hr post-mortem along with freshly filleted, skinned samples prepared from the eviscerated fish. For each sample, *p*H readings were taken at filleting and at skinning.

The iced whole fish captured on August 23 were weighed and examined for *p*H and rigor at killing and at 5, 29, and 53 hr post-mortem. Fillets were prepared from one-third of the lot at each of the above storage periods and stored at 4 C in an airtight assembly which permitted separation of drip from the muscle. Weight, *p*H, and temperature of the fillets were recorded at excising and after 5, 24, 48, 72, and 96 hr storage, and the fillets sampled for EPN immediately on excising and after 24 and 48 hr storage at 4 C.

RESULTS

Studies in 1964 and 1965 confirmed the low *p*H values in trap cod, post-mortem, particularly in fish captured during the 3rd week in July or later when feed was heavy and bottom and surface water temperatures high, about 5 and 10 C respectively (Appendix I). Low *p*H values were associated with rather large amounts of free drip. This feature was common to both sexes (Appendix II). At capture, muscle glycogen in the trap cod was also very high, in support of the data on *p*H and drip levels; all three characteristics however suggested that the poorest fillets will be produced in late midseason (near the end of July).

Weight Changes in Whole Gutted Fish Resulting from Delayed Icing (Table I)

Very small losses to very small gains were registered in the weight of cod not iced at sea (warming in the intervening period to 5, 16, 17, and 20 C), then iced and stored. Maximum losses (0.8%, average) occurred within 29 hr of capture; thereafter, water uptake from the melting ice must have been greater than drip losses. A seasonal effect was noted. All the July 28 fish from a heavily feeding catch taken in warm water and subsequently iced for 15–20 hr lost weight whereas only 4 of the 15 fish taken on June 19 and none of the August 10 fish had lost weight after corresponding periods of storage. Only in the July 28 catch did losses in individual fish extend to 48–53 hr postmortem age.

Weight Changes in Whole Gutted Fish Resulting from Icing at Capture

Rapid chilling to 0.6 C, accomplished in 2 hr or less, resulted in decreased weight losses (Table I). In fact, with the exception of the June 25 samples, the fish iced at sea did not lose weight on the average. Only 10% of the individual samples iced at sea in June, July, and August showed weight losses at 24–29 hr whereas 50% of the samples which were not iced immediately on capture showed losses at the corresponding postmortem period. Only 1 of the 10 fish iced at sea on July 28 showed a weight loss at 24–29 hr. At the same time all 10 fish of July 28, not iced immediately, showed losses.

THE DRIP CHARACTERISTICS OF FILLETS OF TRAP-CAUGHT COD

When handled with normal or less severity, trap fish may develop drip only equalled in other cod exposed to high temperature over a long period. June 25 (item 5, Table II) samples, filleted immediately after killing, produced 3.7% free drip after 24 hr fillet storage (at 4 C). Other trap fish samples (August 10, item 8, Table II) were allowed to warm from 7 C to 17 C in 5 hr, then filleted before rigor mortis. After 5 hr storage at 4 C (average resultant holding temperature 10 C for the whole, warmed fish and the fillets, subsequently chilled to 4 C (Fig. 3A)) the fillets yielded about 3% free drip. This amount is excessive when compared with values for rested Nova Scotia cod live held at 2 C (Dyer and Fraser, 1961). Fillets from the latter cod had to be stored at 18 C for 8 hr to yield 3% drip and produced no measurable amount of of drip when stored at 10 C for 8 hr (Fig. 3B).

MUSCLE GLYCOGEN AT CAPTURE, POSTMORTEM *p*H, FREE DRIP, AND TEXTURE ASSOCIATED WITH SEASON OF CATCH

In 1965 there was a clear seasonal pattern in muscle glycogen levels (Table III). June fish only recently feeding and captured in relatively cold water had an average value of 0.06% ± 0.007 (78 samples). Sixty-eight of

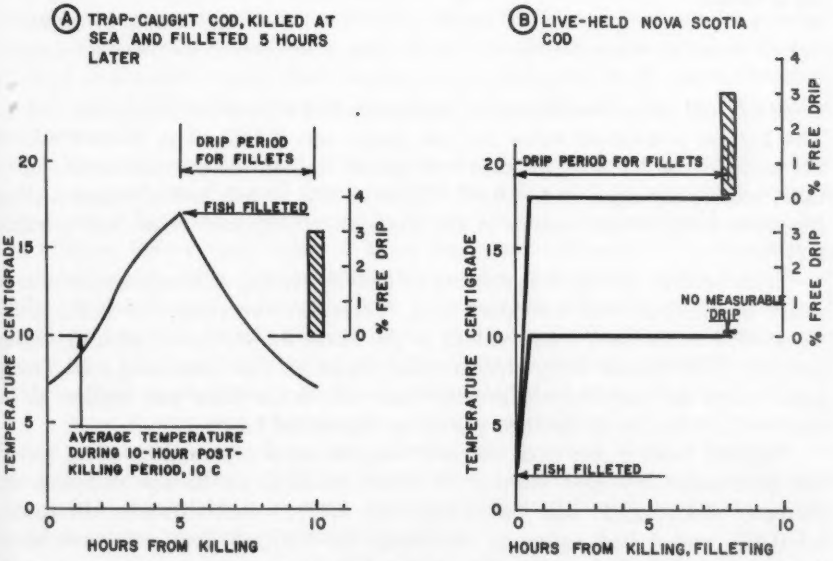

FIG. 3. Handling temperatures and storage times (A) as fish and as fish fillets and (B) as fillets only, associated with the production of free drip in fillets. Samples (A): August 10 trap-caught cod; samples (B): cold, non-feeding tank cod (after Dyer and Fraser, 1961). The trap cod, taken in water at 7 C, warmed to 17 C before they were filleted 5 hr later. The tank fish were at 2 C when filleted immediately after killing.

TABLE III. Feed, muscle glycogen at killing, *p*H drop post-mortem, and organoleptically scored texture values for 1965 trap cod.

	Date of catch			
	June 21	June 21	July 19	August 2
No. of fish	78	10	15	24
Handling ante-mortem	Normal	Very gentle	Normal	Normal
Average no. of capelin found in stomach	3	3	5	1
Average muscle glycogen at killing	0.06%	0.14%	0.50%	0.36%
Range	0.008–0.323%	0.035–0.273%	0.108–1.127%	0.022–0.976%
No. of samples	78	10	15	24
SE	±0.007	±0.021	±0.074	±0.051
Average texture score for fish frozen during rigor	69.0	–	46.3	50.9
No. of samples	309	–	116	138
SE	±0.78	–	±1.10	±1.25
Average drop in *p*H up to 29 hr post-mortem in iced, eviscerated fish	0.20	–	0.67	0.55
No. of samples	78	–	15	24
SE	±0.03	–	±0.03	±0.03

these fish had values less than 0.1% and seven had values in the range 0.1–0.2%. The highest individual value for this group was 0.32%. The 10 live fish of the same lots which were handled very gently had on the average significantly more muscle glycogen (0.14 ±0.02). Three of the 10 fish had glycogen values less than 0.1%, 6 had values in the range 0.1–0.2%, and 1 fish had a value 0.27%.

The average glycogen content of 15 heavily feeding fish captured in warm water in mid-July was 0.50% ±0.07%. Values in the range 0.1–0.5% were found in 8 of the fish, 5 had values in the range 0.5–0.7%, 1 had a glycogen content of 0.9%, and 1.1% glycogen was found in the remaining fish. These high values are significantly greater than values for June fish brailed as in commercial practice or handled gently as individual fish.

Lightly feeding fish from an early August catch captured in warm water had an average glycogen content of 0.36% ±0.05%. Of 24 fish sampled for glycogen at killing, 11 had values less than 0.3%, 9 had values in the range 0.3–0.6%, and 4 had values in the range 0.6–1.0%. Values are lower than those recorded for July fish although the difference is not significant. August fish had passed through the period of heavy feeding and although feed intake was now quite light the results show that they still maintain a high potential energy level.

When compared with glycogen values of 0.01–0.06% for nutritionally fit cod caught by hand line in July and August in the Bay of Fundy (MacLeod and Simpson, 1927), the above data show Newfoundland trap-caught cod obtained in all seasons to be unusually high in muscle glycogen at killing.

The drop in *p*H, up to 29 hr post-mortem, in these iced, eviscerated fish varied throughout the season. The average drop of 0.20, 0.67, and 0.55 *p*H units for June 21, July 19, and August 2 catches respectively, show the *p*H post-mortem to be influenced by the amount of muscle glycogen present at killing (Table III). A comparison of individual fish of all catches showed *p*H drop post-mortem and glycogen among some fish to vary greatly. Only in lightly feeding nutritionally fit August fish was good correlation (R = 0.66, df = 10 (Fig. 4)) found between *p*H drop post-mortem and muscle glycogen in individual fish.

The seasonal trend of July glycogen > August glycogen > June glycogen had a parallel in the seasonal pattern of the texture values for the same trap fish after freezing. Average texture scores (Table III) for samples frozen during rigor in June were 69.0 ± 0.78 (309 samples), in July 46.3 ± 1.10 (116 samples), and in August 50.9 ± 1.25 (138 samples) based on a scale of 0 to 100. All these differences were statistically significant. Also, MacCallum et al. (1966) found texture scores in trap fish processed in June 1962 to be higher than in catches processed later in the season, the poorest fish processed being the late July samples. In the same context, Jones (1965a) shows a relationship between toughness and lactic acid content in cod. The present results provide strong support for Jones' data and show how the extreme extrinsic factors of feed and water temperature encountered in July and early August resulted in fish with high initial glycogen, low postmortem *p*H, and poor texture rating on freezing.

Other data on 1964 and 1965 fish brailed from the traps (Table II) show *p*H drop post-mortem and drip produced by fillets also to vary with season. Comparing June and August samples (items 1 and 13, Table II) *p*H (averages) at filleting before rigor (after 5 hr in ice) were 6.90 and 6.71 respectively, and drip produced in fillets stored 24 hr at 4 C were 1.5% and 4.1%. *p*H at filleting after rigor for June and late July samples (items 3 and 6) were 6.75 and 6.40 and drip in fillets stored 48 hr was 6.1 and 8%. After filleting, post-rigor late July and mid-August samples (items 6 and 12) had 6.40 and 6.63 *p*H readings respectively, and drip in fillets stored 24 hr was 6.0 and 3.4%.

Thus, it was seen that muscle glycogen at killing had an effect on *p*H drop post-mortem which in turn affected the amount of drip produced by stored fillets. All these characteristics varied seasonally. Extreme values were associated with late July–early August catches and were supported by low texture scores measured organoleptically.

THE RELATION OF STATE OF RIGOR AT FILLETING TO THE *p*H AND SUBSEQUENT DEVELOPMENT OF DRIP

Figure 5 shows, for samples obtained throughout the season, the interdependence of *p*H at filleting and free drip produced after 24 hr fillet storage.

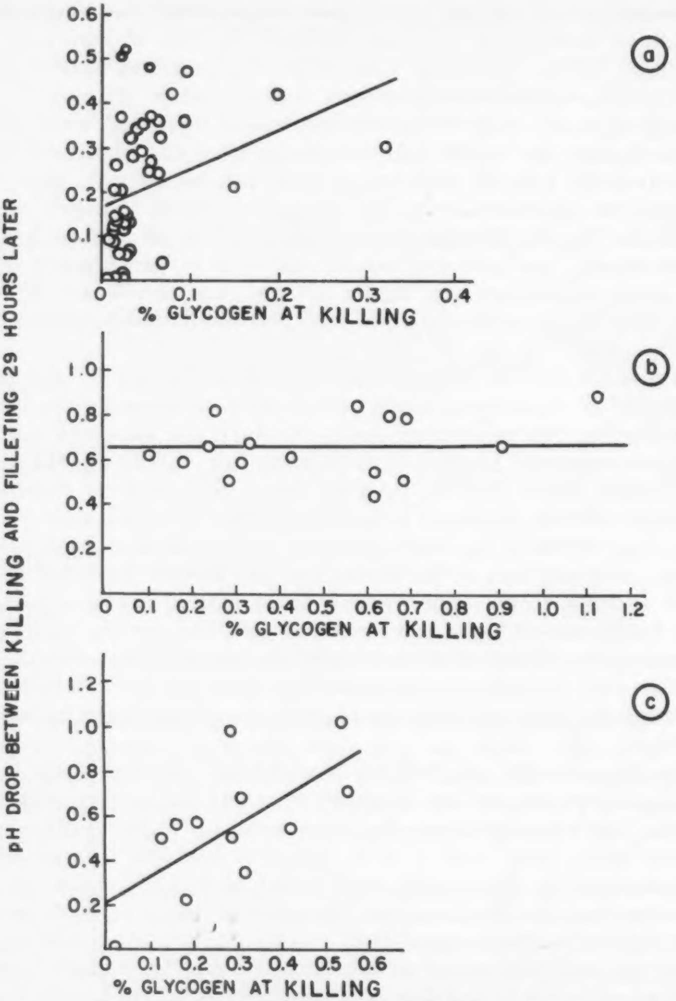

FIG. 4. The regression of *p*H drop (between killing and filleting (during rigor) 29 hr later) on the glycogen in the muscle of the trap-caught fish killed on (*a*) June 21, (*b*) July 19, and (*c*) August 2, 1965.

There was good correlation (R = 0.64, df = 15) between average *p*H of the whole fish at filleting in all stages of rigor and free drip collected from the fillets after 24 hr. On the other hand, it was possible to demonstrate dependence of free drip on the *p*H readings in individual fish only in the cases of pre- (R = 0.71, df = 8) and in-rigor (R = 0.79, df = 8) filleting (August 23 catch, Fig. 6).

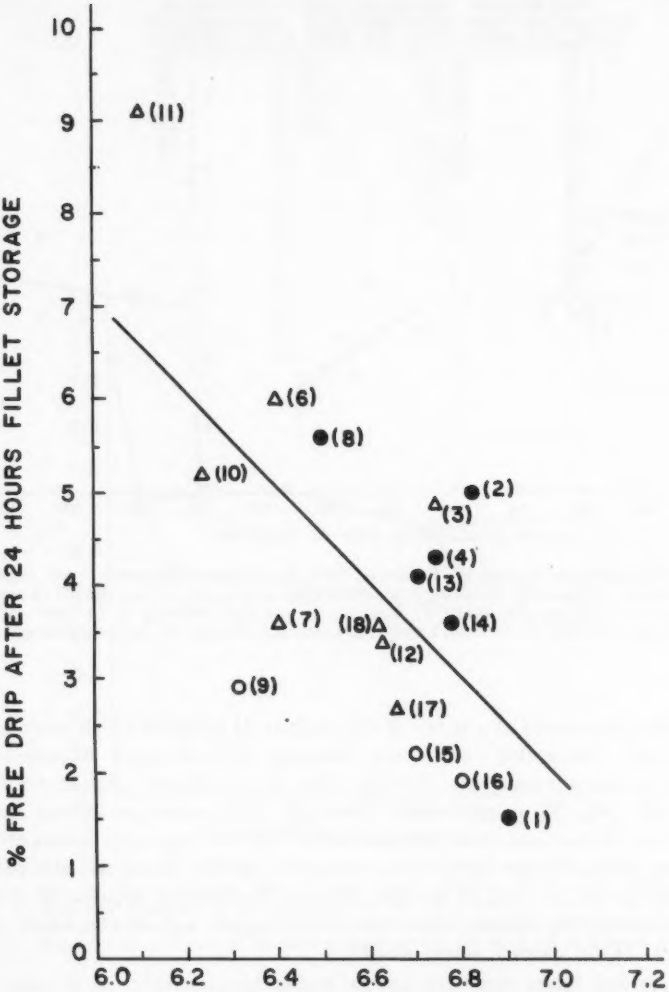

FIG. 5. Relation of *p*H in the whole, eviscerated fish at filleting and the free drip after 24 hr fillet storage. Each point is the average of all the fish from one catch in one state of rigor at filleting. Pre-rigor filleting (●); in-rigor filleting (○); post-rigor filleting (△). Numbers in parentheses refer to items in Table II.

Thus, in general, in-rigor filleting resulted in lowest drip but the varied handling associated with the samples illustrated in Table II and in Fig. 5 does not warrant drawing more precise conclusions. Such, however, may be

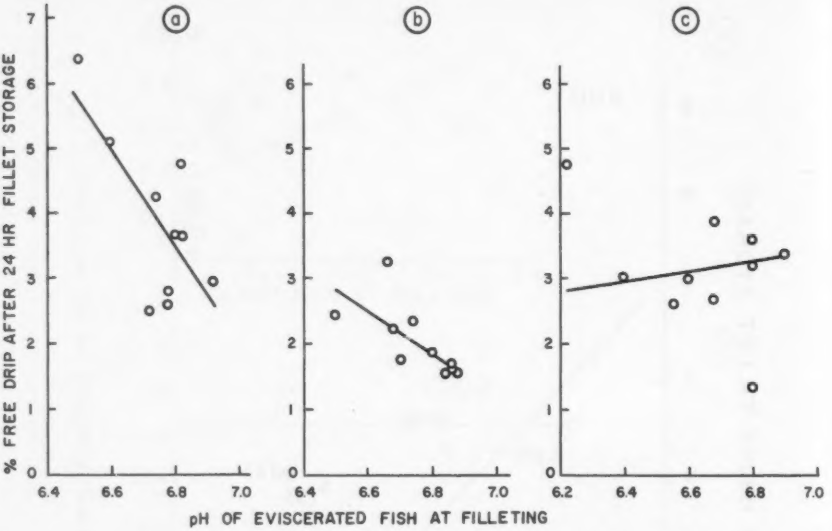

FIG. 6. The regression (George W. Snedecor, 1956, Iowa State University Press, Ames, Iowa, Chap. 6, 5th ed.) of free drip (resulting from 24 hr fillet storage at 4 C) on the *p*H of eviscerated trap-caught cod (August 23 catch) at filleting: *a*, pre-rigor filleting (5 hr from killing); *b*, in-rigor filleting (29 hr from killing); *c*, post-rigor filleting (53 hr from killing).

drawn from the results of a study of the August 23 samples which were handled under closely controlled conditions. Samples of this catch filleted in-rigor produced minimum amounts of drip. The fillets showed almost no further drop in *p*H (Fig. 7) in contrast to pre-rigor and post-rigor filleted samples. At least two phenomena could be responsible for the large drip values obtained from fillets prepared pre-rigor, the contraction of the fillets and the *p*H drop after filleting. In the case of in-rigor filleting the smaller amount of drip was not counteracted by weight losses before filleting — indeed the whole gutted fish stored 24 hr gained some weight.

In handling fillets that will not be frozen immediately (as in some commercial practice), the advantage of filleting during rigor rather than before is further illustrated by the data on drip reported in Table II for the August 10 catch. Fillets excised from fish in full rigor (item 9) developed about half the drip obtained from fillets removed from the warm, pre-rigor cod (item 8).

Thus, to minimize drip development, fish that are permitted to proceed into rigor before freezing should undergo this change in the whole, gutted state, rather than as fillets. In fish so iced the skeleton is called upon to resist the contractive forces of rigor (Love, 1962; Jones, 1965b) and the fillets, when excised, release a minimum amount of drip.

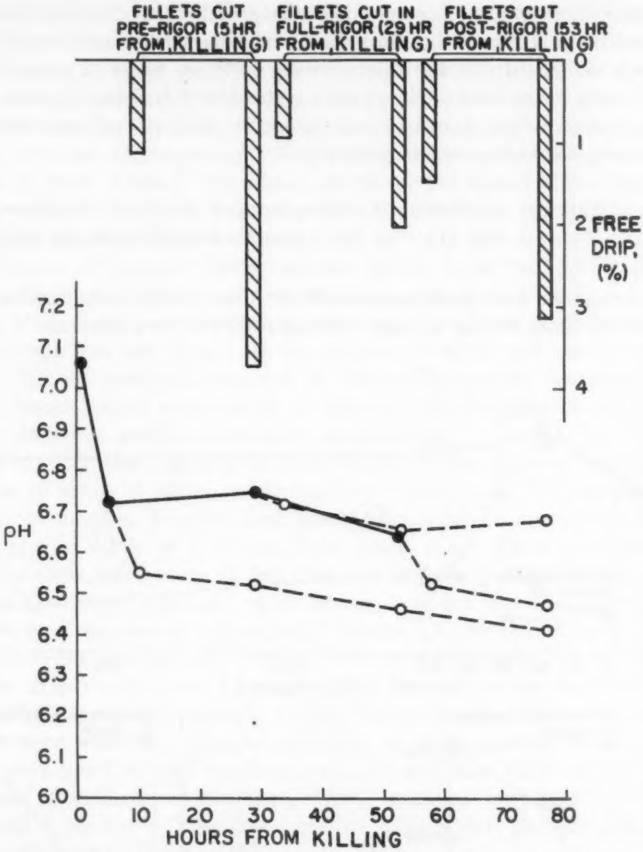

Fig. 7. The *p*H of iced, whole cod captured on August 23 (solid lines) and of fillets of these fish (broken lines) prepared 5 hr after capture (pre-rigor filleting), 29 hr after capture (in-rigor filleting), and 53 hr after capture (post-rigor filleting). The inverted bar chart shows the percentage free drip after 5 and 24 hr fillet storage at 4 C for each lot of filleted fish.

Effect of Temperature Before and Subsequent to Filleting on the Development of Free Drip

Although environmental factors of feed and seawater temperature correlate with unusually high amounts of drip developed in fillets of trap fish, chilling the unfilleted material from capture greatly reduces the amount of drip formed. Lightly feeding fish caught on August 23, iced constantly for 5 hr after capture, filleted before rigor, and then held at 4 C for another 5 hr (items 13 and 14, Table II) produced about 1% drip in comparison with approximately 3% for August 10 samples warmed to 17 C before filleting and subsequently chilled to 4 C (item 8). Continuous chilling of heavily feeding, July 28 fish from time of gutting at the traps until filleting after rigor mortis (item 7) resulted in 3.6% drip in the fillets versus 6.0% in fillets of other fish (item 6) of the same catch held without ice for the first 9 hr after capture (to simulate some commercial practice) and thereafter iced, stored, and filleted along with the samples continuously under ice.

THE CONCOMITANT ASPECTS OF INSOLUBILITY OF THE PROTEINS, TASTE-PANEL RATING, AND DRIP IN RELATION TO STATE OF RIGOR AT FILLETING (FIG. 8)

In agreement with results reported by Love (1962) and Partmann (1960) it was found that, during storage, the solubility of the proteins in post-rigor

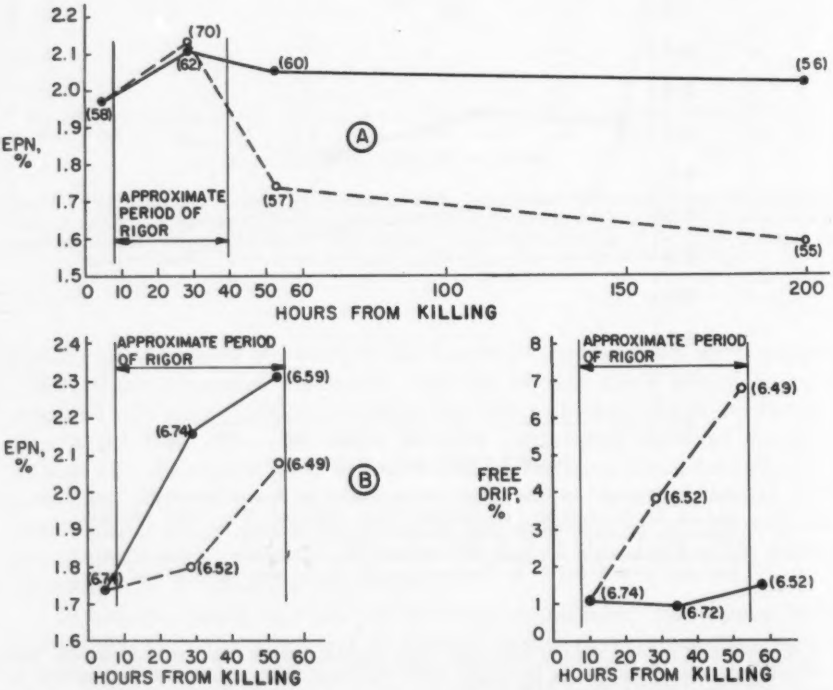

FIG. 8. EPN — time curves for eviscerated trap cod and fillets held unfrozen. A, August 17 catch; fillets removed from iced, eviscerated fish and analyzed immediately (solid line) and fillets prepared before rigor mortis, 5 hr after killing (broken line) and analyzed periodically after iced storage. Texture ratings (taste-panel assessment) are shown in parentheses on the curves. B, August 23 catch; fillets removed from iced, eviscerated fish and analyzed immediately (solid line) and fillets prepared before rigor, 5 hr after killing (broken line) and analyzed periodically after chill storage at 4 C. Also, for the August 23 catch, drip values for fillets prepared at three stages of rigor were recorded after 5 hr fillet storage (solid line) respectively, and for those filleted before rigor after additional storage for 24 and 48 hr (broken line). The *p*H of the fillets is shown in parentheses on both sets of curves.

excised (August 17 catch) fillets of iced trap fish was considerably higher (EPN > 2.0) than in stored, iced fillets (EPN 1.6 and 1.7) excised before the development of rigor mortis. However, organoleptic scores for the texture of fillets (both immediately after processing and after storage after pre-rigor filleting) were uniformly fair, showing no correlation with changes in EPN except in the case of the stored fillets during the period of development and resolution of rigor. During this period of change the stored fillets first showed increased ratings for both texture and EPN and, as rigor was resolved, dropped precipitously on both counts.

On August 23, stored fillets (excised before rigor) had about 0.3 units less salt soluble protein when measured during the period of rigor resolution than had fillets freshly excised from eviscerated iced fish of the same catch. This difference was not shown in the August 17 data and would indicate a preference for chill-storing trap fish in the whole gutted condition if they cannot be frozen within a few hours of capture, a finding similar to that noted when free drip was used as a measure of quality.

Some concomitant aspects of insolubility of the proteins and free drip in relation to state of rigor at filleting were also noted in connection with the August 23 samples. At 29 hr, iced, whole gutted fish in rigor mortis produced fillets having an EPN of 2.16 and, 5 hr later, these fillets had yielded 1% drip. At the same time, fillets of fish that had been prepared 5 hr after capture had an EPN value of 1.80 and, upon storage, yielded 4% drip. Fifty-three hr after capture corresponding values for EPN were 2.32 for the fillets immediately removed from the iced fish and 2.08 for fillets prepared only 5 hr after capture. Respective drip values were 1.5 and 6.8%. The *p*H of the samples, filleted before rigor, dropped significantly in relation to samples filleted during rigor. Thus, the data associate reduced solubility of the proteins (in 0.6 M NaCl solution) with low *p*H amd maximum drip in unfrozen fillets of cod prepared before rigor. Tomlinson et al. (1965, 1966), working with iced halibut (post-rigor) found a similar relationship between muscle *p*H, protein denaturation (1965), and free drip (1966).

THE VARIABILITY IN INITIAL CONDITION OF TRAP FISH

Three of five cod captured on August 10, non-iced for 5 hr, then iced for 24 hr were in firm rigor (item 9, Table II) at the end of that time. Rigor was partly resolved in the fourth (item 10, Table II) and fully resolved in the fifth (item 11, Table II) fish. The fillets from the three stiff fish (curves N, P, Q, Fig. 9) developed considerably less than half the drip collected from the fifth sample (curve L) and about half that collected from the fourth fish (curve M).

The figure confirms the conclusions reached earlier, namely that highest drip values are associated with fillets of lowest *p*H. However, in addition it shows that the fluid-binding capacity of fillets of trap cod which have been iced for 24 hr has no constant relation to the period of iced storage; the state of rigor and the *p*H at filleting would seem to be important criteria.

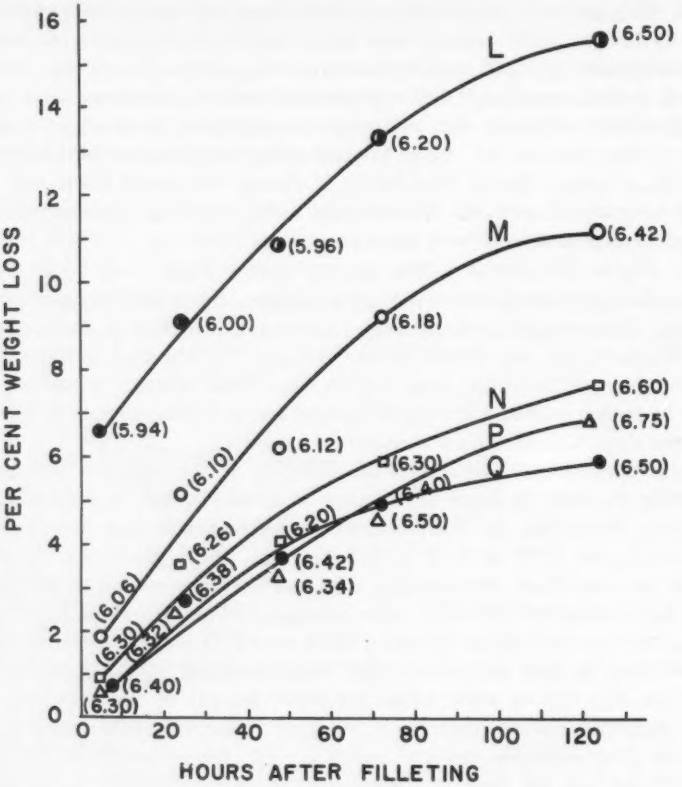

FIG. 9. Weight loss in each of five pairs of fillets (stored at 4 C) cut from fish of the same catch (August 10). Although the fish were given the same pre-filleting treatment (5 hr not iced, 24 hr iced storage), at filleting three of the fish (N, P, Q) were in rigor, one partly out of rigor (M), and one completely out of rigor (L). The *p*H of the fillets is shown in parentheses on the curves.

CONCLUSIONS AND RECOMMENDATIONS

The results obtained throughout the trap-fishing season show the interdependence of four factors — seawater temperature, feed, free drip, and *p*H, the first two being apparently causitive with respect to free drip and *p*H development. The amount of free drip in fillets increased in proportion to the drop in *p*H of the whole eviscerated fish used for filleting. The quantity of drip and *p*H depression post-mortem may be controlled in some measure by judicious postmortem handling and by filleting at selected states of rigor. Thus, in the trap fish: (1) June seawater temperatures < temperatures after about August 6 < temperatures in the period ranging from approximately July 8 to August 4 (the latter relation in 1964 only); (2) feed (and muscle glycogen at capture) for early June fish < August fish < July fish; (3) free drip from June samples < August samples < July samples; and (4) *p*H post-mortem in June samples > August samples > July samples. As seen from the above, the increased feed and water temperatures in the late July to early August period were the precursors of maximum drip development and *p*H depression post-mortem. Studies after freezing also showed the texture of these late July catches to be poorer than that of samples prepared earlier and later in the season, similar to a recently published finding in studies on 1962 catches of trap cod.

In individual catches, the low levels of *p*H in eviscerated fish were maintained throughout the various states of rigor. But after filleting (before and after rigor) there was a further drop in *p*H and in some fillets prepared before rigor a loss in solubility of the proteins in 0.6 M NaCl solution. The *p*H of fillets processed before rigor was significantly lower and the amount of free drip formed significantly greater than in fillets prepared during rigor. The time the whole fish remained in ice was not always found to be the controlling factor in drip production. Because of differences between individual fish the state of rigor and the *p*H at filleting are the important criteria.

Thus, the deleterious factors of free drip, decreased *p*H, and low EPN values were found to be most pronounced in fillets cut from pre-rigor fish and thereafter stored at above freezing temperature. Therefore, it is suggested that trap fish held for many hours before freezing, as required in some processing centres, be chilled meanwhile and stored in the eviscerated state and *not* held as unfrozen fillets.

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REFERENCES

BANKS, A. 1955. The expressible fluid of fish fillets. IV — The expressible fluid of iced cod. J. Sci. Food Agr., **6**: 584–587.

CARROLL, N. V., R. W. LONGLEY, AND J. W. ROE. 1956. The determination of glycogen in liver and muscle by use of amthrone reagent. J. Biol. Chem., **220**: 583–593.

CUTTING, C. L. 1951. Loss of weight and shrinkage of iced fish on trawlers. Fish. News, No. 1975. p. 10. (DSIR Food Invest. Mem. No. 701.)

Dyer, W. J., H. Brockerhoff, R. J. Hoyle, and Doris I. Fraser. 1964. Polyphosphate treatment of frozen cod. I. Protein extractability and lipid hydrolysis. J. Fish. Res. Bd. Canada, **21**(1): 101–106.

Dyer, W. J., and F. E. Dyer. 1947. Losses through the leaching by water from melting ice of soluble constituents in iced, gutted cod. Progr. Rept. Atlantic Coast Sta., Fish. Res. Bd. Canada, No. 40, Note No. 95. p. 3–5.

Dyer, W. J., and Doris I. Fraser. 1961. Moisture in fish blocks processed from very fresh fish. Canadian Fisherman, **48**(8): 17–19.

Dyer, W. J., H. V. French, and J. M. Snow. 1950. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. J. Fish. Res. Bd. Canada, **7**(10): 585–593.

Ellison, W. A., Jr. 1934. The prevention of spoilage at sea. Fish. Gaz., **51**: 17.

Fraser, Doris I., H. M. Weinstein, and W. J. Dyer. 1965. Postmortem glycolytic and associated changes in the muscle of trap- and trawl-caught cod. J. Fish. Res. Bd. Canada, **22**(1): 83-100.

Jean, Yves. 1964. Seasonal distribution of cod (*Gadus morhua* L.) along the Canadian Atlantic Coast in relation to water temperatures. Ibid., **21**(3): 429–460.

Jones, N. R. 1965a. Problems associated with freezing very fresh fish. *In* Fish handling and preservation. Organisation for Economic Co-Operation and Development, Paris. p. 31.

1965b. Freezing fillets at sea. *In* World Fishing and The White Fish Authority [ed.] Fish quality at sea. Grampian Press Ltd., London.

Love, R. M. 1962. Protein denaturation in frozen fish. VII. Effect of the onset and resolution of rigor mortis on denaturation. J. Sci. Food Agr., **13**: 534–545.

MacCallum, W. A., E. J. Laishley, W. J. Dyer, and D. R. Idler. 1966. Taste panel assessment of cod fillets after single and double freezing. J. Fish. Res. Bd. Canada, **23**(7): 1063–1081.

MacLeod, J. J. R., and W. W. Simpson. 1927. The immediate post mortem changes in fish muscle. Contrib. Canadian Biol. Fish., **3**: 437–456.

MacPherson, N. L. 1932. Studies in the behaviour of the carbohydrates and lactic acid of the muscle of haddock after death. Biochem. J., **26**: 80–87.

Montgomery, R. 1957. Determination of glycogen. Arch. Biochem. Biophys., **67**: 378–386.

Partmann, Walter. 1960. Rigor und Gefrierveranderungen bei Susswasserfischen un Abhangigkeit von der Zeit post mortem. Arch. Fischereiwiss., **11**: 81.

1964. Changes in proteins, nucleotides and carbohydrates during rigor mortis. *In* Rudolf Kreuzer [ed.] Technology of fish utilization. Fishing News (Books) Ltd., London. p. 4–13.

Snow, J. M. 1950. Proteins in fish muscle. II. Colorimetric estimation of fish muscle protein. J. Fish. Res. Bd. Canada, **7**(10): 594–598.

Templeman, W., and A. M. Fleming. 1956. The Bonavista longlining experiment, 1950–1953. Bull. Fish. Res. Bd. Canada, No. 109.

1962. Cod tagging in the Newfoundland area during 1947 and 1948. J. Fish. Res. Bd. Canada, **19**(3): 445–487.

Templeman, W., and A. W. May. 1964. Research vessel catches of cod in the Hamilton Inlet Bank area in relation to depth and temperature. Intern. Comm. Northwest Atlantic Fish. Spec. Publ. No. 6. p. 149–165.

Tomlinson, N., S. E. Geiger, and E. Dollinger. 1965. Chalkiness in halibut in relation to muscle *p*H and protein denaturation. J. Fish. Res. Bd. Canada, **22**(3): 653–663.

1966. Free drip, flesh *p*H, and chalkiness in halibut. Ibid., **23**(5): 673–680.

APPENDIX I

SEASONAL CHANGES in SEAWATER TEMPERATURE at the TRAPS

Bottom and surface temperatures encountered during the 1964 and 1965 seasons are listed in Table IV. In the 2 years, temperatures gradually increased up to early August. Drastic changes in water temperature occurred within short periods, i.e. July 28–29, 1964 and August 2–6, 1965. The same trap was visited on August 5 and 6, 1965. The catches (ungutted weight) from these particular hauls amounted to 2600 and 2000 lb respectively. The cold water of August 5, 1965 followed a 2-day period of offshore winds. The return of warmer water on August 6 was accompanied by a drop in these winds, a change to low velocity south westerlies, and a calmer sea. Maximum and

TABLE IV. Bottom (at depths varying from 8 to 18 fath) and surface temperature alongside traps.

Date	Temperature (*C*)	
	Bottom	Surface
1964 Season		
June 15	−0.7	3.0
19	1.2	5.0
25	−1.1	3.1
July 8	2.5	6.5
14	1.1	7.5
21	–	9.2
28	2.2	2.6
29	6.6	10.3
Aug. 3	8.0	11.2
4	1.3	11.5
6	0.6	7.2
10	0.9	5.2
1965 Season		
June 4	1.5	2.2
21	3.3	4.5
23	5.0	5.9
24	5.8	6.1
July 19	5.0	9.7
Aug. 2	5.4	12.4
5	0.4	2.2
6	2.6	11.6
10	3.9	9.8
18	1.0	10.7
23	–	14.0

minimum air temperatures on August 4 and 5 were approximately 70 and 54, and on August 6, 78 and 54. Thus, the study shows that cod are trapped in relatively warm water at the end of the fishing season. At this time the feed is light compared to that found 3 or 4 weeks earlier. Surveys show that cod normally prefer a colder environment (Jean, 1964; Templeman and May, 1964) but may be fished inshore as in the Hamilton Inlet area at variable temperatures with a range of at least −0.8 to 8.7 C (Templeman and May, 1964). The range of temperatures shown in Table IV is only slightly greater than the above and the variability of temperatures suggested by Templeman and May (1964) is confirmed.

APPENDIX II

THE EFFECT OF THE SEX OF THE CATCH ON *p*H AND DRIP DEVELOPMENT AND ON THE SOLUBILITY OF THE PROTEINS

In all examinations at the trap there was close to a 1:1 relation between numbers of fish of the two sexes. Equal numbers of male and female fish of the August 23 catch were treated in the same manner. There were no significant differences in *p*H values, drip produced, or salt soluble protein values which could be related to sex. From this it is assumed that the particular sex of none of the other catches reported was a contributing factor to differences found in dissimilarly treated fish.

A Tumorlike Growth on the Foot of a Freshwater Mussel (*Anodonta californiensis*)[1,2]

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ABSTRACT

A freshwater mussel, *Anodonta californiensis*, was found which possessed a small tumorlike growth on the foot that measured 3 mm in diameter and 2 mm in height. This abnormal growth, thought to be an adenomyoma, was located laterally near the ventral edge of the foot. The growth consisted of dark basophilic glandular cells and muscle fibers with a deeply convoluted epithelial covering. The cause of the lesion is not known.

INTRODUCTION

Tumors are a rare occurrence among bivalve molluscs. Williams (1890) reported a pedunculated tumor, composed of glandular and muscle cells, on a freshwater mussel, *Anodonta cygnea*. Collinge (1891) also observed two tumors from the same species of freshwater mussel, but gave no microscopic description. These three tumors were all apparently outgrowths of the mantle. A connective tissue tumor arising from the palps of *A. implicata* was described by Butros (1948). Mesenchymal tumors have been reported originating from the pericardial cavity of eastern oysters, *Crassostrea virginica*, (Ryder, 1887; Smith, 1934) and the rectum of a Pacific oyster, *C. gigas* (Sparks et al., 1964a). Hueper (1963) noted several papillary tumors around the rectum of soft-shelled clams, *Mya arenaria*, and presented excellent photographs of the gross lesions, but gave no microscopic discussion. A tumorlike mass, which was the rectum expanded due to a fecal impaction, was also reported by Sparks et al. (1964b).

During a routine monthly sampling of a population of Columbia River freshwater mussels (*A. californiensis* and *A. oregonensis*) for glycogen analysis, a mature male *A. californiensis* was observed to possess a small tumorlike growth, located laterally near the ventral edge of the foot. A description of this lesion seems appropriate due to its unique location and the dearth of histochemical observations about molluscan neoplasms.

MATERIALS AND METHODS

The freshwater mussel was examined grossly and fixed in 10% calcium-formal fixative. The tissues were embedded in Paraplast and sections were made through the abnormal growth to determine its microscopic structure.

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[2] This paper is based on work performed under the United States Atomic Energy Commission Contract AT(45-1)-1830.

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The tissue sections were stained with Harris' hematoxylin and eosin or Clarke's PAS reagent. To help localize the glycogen, tissue sections treated with 1% diastase were utilized as controls, since diastase-labile portions of PAS-positive cells are interpreted to represent glycogen. Clarke's procedure (Clarke, 1965) was modified for the freshwater mussels by under-staining in the PAS reagent and over-treating in the diastase because of the high affinity of this animal's tissue for the PAS solution.

RESULTS

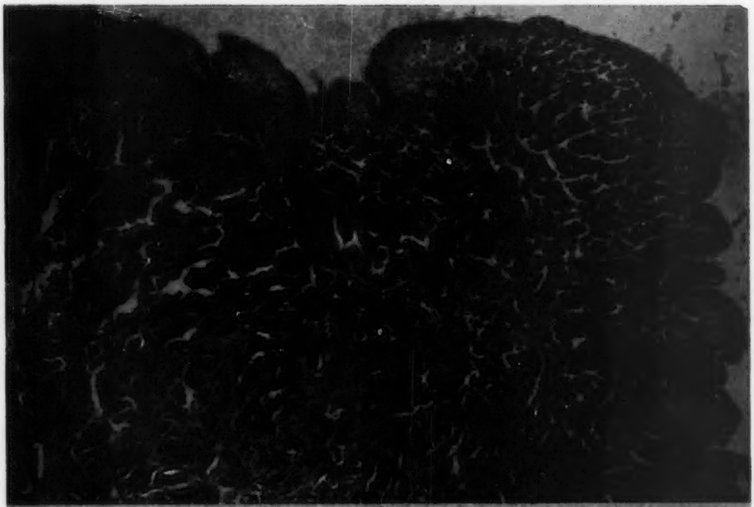
Upon gross examination, the tumorlike growth was a small, firm papilliform projection of the foot. The abnormal growth was located laterally near the ventral edge of the foot and measured 3 mm in diameter and 2 mm in height.

The lesion was covered by a convoluted, vacuolated epithelium with faded cytoplasm (Fig. 1) that was strikingly different from the normally tall columnar epithelium of the foot (Fig. 3). The stroma of the tumor consisted of deeply basophilic glandular cells and muscle fibers (Fig. 4). The epithelial covering was conspicuously convoluted with deep crypts that penetrated into the base of the lesion (Fig. 5). The basophilic cells of the tumor appeared similar to those found just beneath the normal epithelium of the foot, but differed from the normal glandular cells in that they were arranged irregularly. The muscle fibers of the abnormal growth also differed from the normal muscle of the foot by not being arranged in large orderly muscle bundles. Among the muscle and gland cells of the tumor, a few small, yellow cells, classified as leucocytes, were present, but no heavy infiltration of leucocytes was observed. The neoplasm was apparently benign since it was well delimited and did not appear to be invasive into the normal tissue.

The distribution of glycogen in the tumorlike growth showed no apparent abnormality (Fig. 2 and 6). The muscle fibers of the lesion had fine granular deposits of glycogen (Fig. 6 and 7) similar to the normal foot muscles. The basophilic glandular cells in the tumor were highly PAS-positive (Fig. 6 and 7), like the glandular cells beneath the normal epithelium. Since these highly PAS-positive cells did not possess glycogen, it is presumed that they contain a mucopolysaccaride.

DISCUSSION

Reviews of molluscan neoplasia show no definite malignant growths have been reported among these animals (Scharrer and Lochhead, 1950; Wautier and Wautier, 1953; Kenton, 1964). Carcinogens have been used on a variety of invertebrates with negative results (Scharrer and Lochhead, 1950), but have induced tumors in cephalopods, *Sepia* sp., (Jaquemain et al., 1947). Since the area occupied by the tumor of *A. californiensis* was well defined with no evidence of invasion into the surrounding normal tissue, this lesion was considered to be a benign growth. Therefore, following vertebrate terminology, this benign tumor consisting of glandular and muscle cells could be termed an adenomyoma.



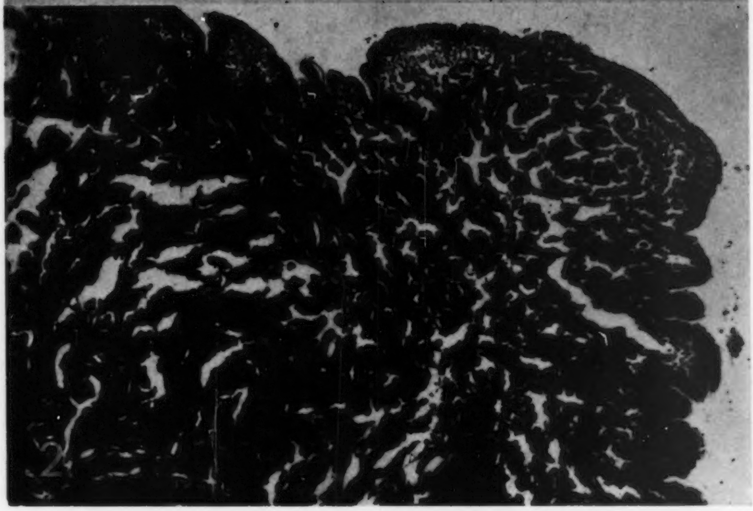


FIG. 1. Benign turmorlike growth on the foot of a freshwater mussel. Normal muscles of the foot are visible at lower left. Hematoxylin and eosin. 30×.

FIG. 2. Tumorlike growth on the foot of a freshwater mussel, stained for glycogen. Note intense PAS-positive reaction of both the tumor and the normal muscles of the foot. Clarke's PAS. 30×.

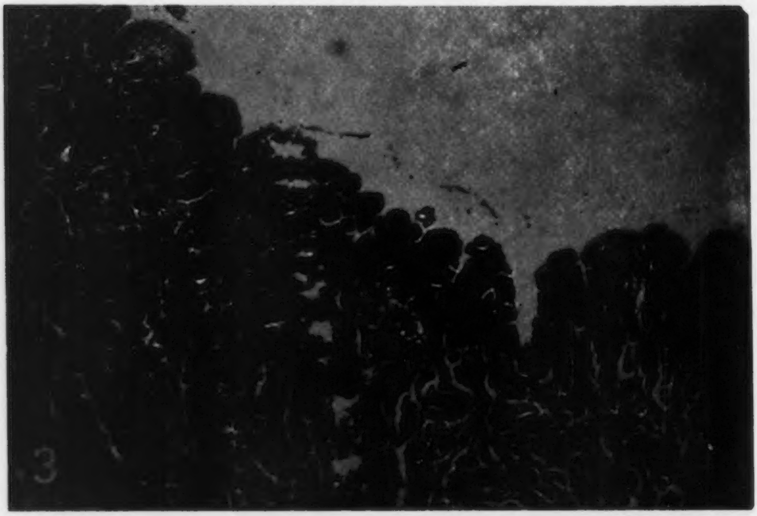


FIG. 3. Foot of freshwater mussel, showing part of the abnormal growth at left and normal epithelium of the foot at right. Hematoxylin and eosin. 30×.

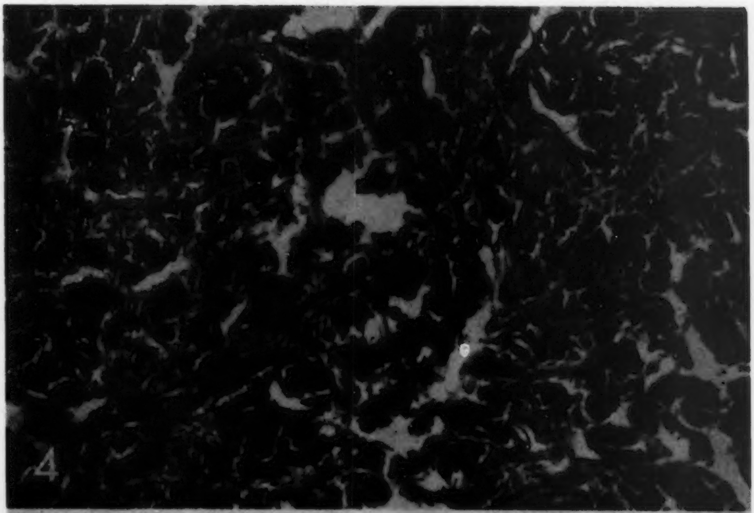


FIG. 4. Higher magnification of freshwater mussel lesion. Note the deeply basophilic cells and the muscle fibers that make up the majority of the tumor. Hematoxylin and eosin. 155×.

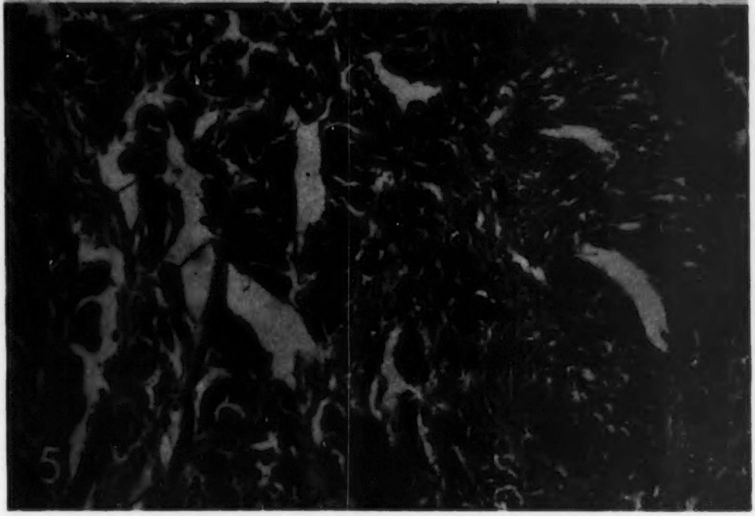
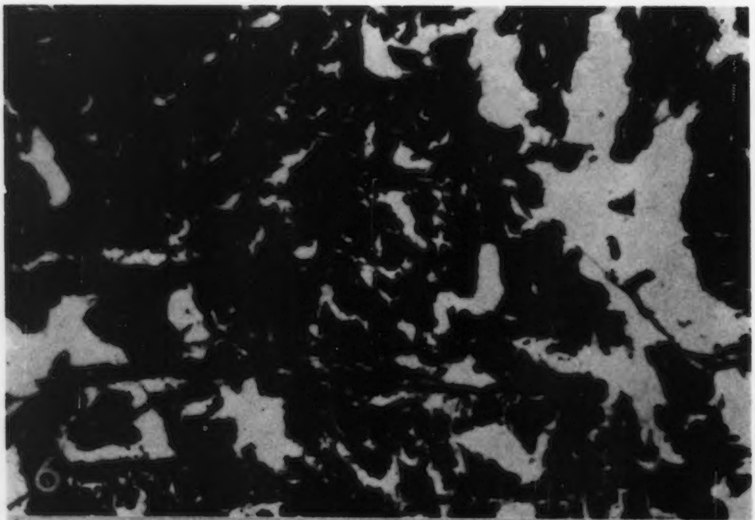


FIG. 5. Tumorlike growth on freshwater mussel, showing deep epithelial crypt at right and located near the base of the tumor. Hematoxylin and eosin. 155×.



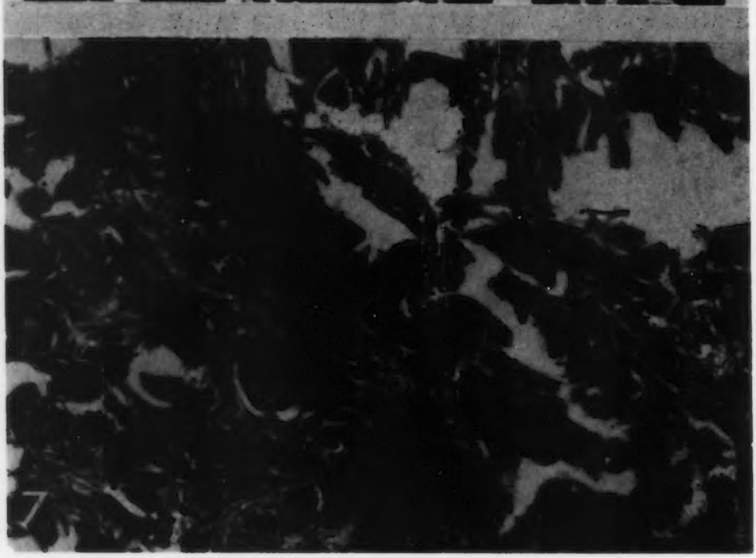


FIG. 6. Freshwater mussel lesion stained for glycogen. Note intense PAS-positive reaction of both the glandular cells and the muscle fibers. Clarke's PAS. 155×.

FIG. 7. Abnormal growth stained for glycogen and treated with diastase. Note that the muscle fibers possessed PAS-positive material (Fig. 6) that was diastase labile, whereas the glandular cells remained PAS-positive after diastase treatment. Clarke's PAS–1% diastase. 155×.

However, Scharrer and Lochhead (1950) state that terminology developed almost exclusively for use in mammalian pathology should not be applied to invertebrate animals.

The tumor on *A. californiensis* differed from previous tumor reports among bivalves by its unique location and small size, its lack of a stalk, and its microscopic structure. Collinge (1891) did report an unstalked tumor, but gave no microscopic description. Williams (1890) reported an adenomyoma in *A. cygnea*, with the glandular cells just beneath the epithelium and entirely separated from the muscular stalk or core. The growth reported by Williams (1890) would appear to closely resemble the pedunculated adenoma, or polyp, commonly found in the human colon (Robbins, 1962). The lesion on *A. californiensis* reported here in no way resembles a polyp; hence the name adenomyoma, rather than pedunculated adenoma.

The large tumors reported in *A. implicata* (Butros, 1948), *C. virginica* (Smith, 1934), and *C. gigas* (Sparks et al., 1964a) possessed a marked inflammation. A few cells resembling leucocytes were found throughout the tumor in *A. californiensis*, but these cells differed from any of the blood cell descriptions for freshwater mussels given by Dundee (1953). Shallow convolutions on the surface of molluscan tumors have been reported by Butros (1948) and Smith (1934). Sparks et al. (1964a) noted deep epithelial folds or crypts on a molluscan neoplasm. However, none of these crypts penetrated into the base of the tumor as deeply as those in the lesion on *A. californiensis*. The microscopical comparisons of these tumors indicate that the adenomyoma found on the foot of *A. californiensis* is uniquely different from molluscan neoplams reported previously.

Most of the tumors recorded from molluscs have been large, plainly visible neoplasms. The small size of the growth on the foot of *A. californiensis* suggests that neoplasms might be observed more commonly among molluscs if more effort and time were spent examining shellfish populations for tumorous growths. It is possible that neoplasms are more common than the literature indicates, since Hueper (1963) found 2% of a population of *M. arenaria* possessing rectal papillomas. However, Williams (1890) found only one tumor after examining 700 freshwater mussels, *A. cygnea*, and no neoplasms were observed by Taylor (1966) after examining 1114 California mussels, *Mytilus californianus*, and 1000 bay mussels, *M. edulis*, for possible tumorous growths.

This tumor may be considered as a spontaneous growth (Scharrer and Lochhead, 1950) since its cause is unknown. As pointed out by Sparks et al. (1964), it is possible that tumors in molluscs are not neoplasms as the word is used in vertebrate pathology, and they have resulted as a response to injury or infection. There is a possibility that the tumorlike growth on the foot of *A. californiensis* is not a true neoplasm, but a hyperplasia of the basophilic and muscle cells beneath the epithelium of the foot, since the cells within this lesion all appeared normal with both hematoxylin and eosin stain and Clarke's PAS reagent. If this abnormal growth is a hyperplasia, it may have been produced by some type of irritation or injury. Many investigators have reported invertebrate tumors which are in reality wound responses (Scharrer and Lochhead, 1950), and tumefactions (swellings) occur in molluscs due to infection (Taylor, 1966). The origin and classification of molluscan tumors will remain uncertain until more of these abnormal growths are studied and the processes of injury and infection among molluscs are thoroughly understood.

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REFERENCES

Butros, J. 1948. A tumor in a freshwater mussel. Cancer Res., **8**: 270–272.

Clarke, W. J. 1965. Unpublished procedure. Pathol. Dept., Battelle-Northwest, Richland, Wash.

Collinge, W. E. 1891. Note on a tumor in *Anodonta cygnaea* Linn. J. Anat. Physiol. Norm. Pathol., **25**: 154.

Dundee, D. S. 1953. Formed elements of the blood of certain freshwater mussels. Trans. Am. Microscop. Soc., **72**: 254–264.

Hueper, W. C. 1963. Environmental carcinogenesis in man and animals. Ann. N.Y. Acad. Sci., **108**: 963–991.

Jaquemain, R., A. Jullien, and R. Noel. 1947. Sur l'action de certains corps cancérigènes chez les céphalopodes. Compt. Rend., **225**: 441–443.

Kenton, Charlotte. 1964. Tumors in invertebrates: a bibliography of reports on neoplasia of invertebrates, 1950–1963. Unpubl. Rept., Med. Library, Natl. Inst. Health. 19 p.

Robbins, S. L. 1962. Textbook of pathology with clinical application. 2nd ed. W. B. Sanders Comp., Philadelphia. p. 693–695.

Ryder, J. A. 1887. On a tumor in the oyster. Proc. Acad. Nat. Sci. Phila., **44**: 25–27.

Scharrer, B., and M. S. Lochhead. 1950. Tumors in the invertebrates: a review. Cancer Res., **10**: 403–419.

Smith, G. M. 1934. A mesenchymal tumor in an oyster (*Ostrea virginica*). Am. J. Cancer, **22**: 838–841.

Sparks, A. K., G. B. Pauley, R. R. Bates, and C. S. Sayce. 1964a. A mesenchymal tumor in a Pacific oyster, *Crassostrea gigas* (Thunberg). J. Insect Pathol., **6**: 448–452.
1964b. A tumorlike fecal impaction in a Pacific oyster, *Crassostrea gigas* (Thunberg). J. Insect Pathol., **6**: 453–456.

Taylor, R. L. 1966. *Haplosporidium tumefacientis* sp. n., the etiologic agent of a disease of the California sea mussel, *Mytilus californianus* Conrad. J. Invertebrate Pathol., **8**: 109–121.

Wautier, V., and J. Wautier. 1953. Le cancer et les invertébrés. Bull. Mens. Soc. Linneenne Lyon, **22**: 67–96.

Williams, J. W. 1890. A tumor in the freshwater mussel (*Anodonta cygnea*) Linn. J. Anat. Physiol. Norm. Pathol., **24**: 307–308.

NOTES

Food of the Dolphin, *Coryphaena hippurus* Linnaeus, and of the Yellowfin Tuna, *Thunnus albacares* (Lowe), from Barbados, West Indies

The dolphin, *Coryphaena hippurus* Linnaeus, and the yellowfin tuna, *Thunnus albacares* (Lowe), are both widely distributed in tropical and subtropical seas. They are well-known food and game fish in the Caribbean. The dolphin is a common food species in Barbados and is second in importance only to the flyingfishes in the local fishing industry (Hall, 1955). The following study is a contribution to the feeding biology of these two species in the southeast Caribbean.

Specimens were obtained during the period February to June only, from commercial catches and from game fishermen. All specimens were caught by trolling with towed bait and were taken within a distance of 10–20 miles from the coast of Barbados. Stomachs were removed as soon as possible and preserved in 5–10% formalin. With the exception of a single juvenile of 10.5 inches, all dolphins were adults between 31 and 49 inches standard length. The tunas were between 36 and 50 inches standard length. Seventy specimens of *C. hippurus* were examined and 12 *T. albacares*.

All stomachs, except those of three dolphins, contained some recognizable food remains. The most important prey of the dolphin were adult flyingfishes (Exocoetidae), which occurred in nearly 60% of all specimens (Table I).

The second most important prey of the dolphin was the flying gurnard, *Dactylopterus volitans*. Juvenile stages of this species occurred in nearly 60% of the stomachs examined.

Three other groups formed important elements in the diet of *C. hippurus*. These were the filefishes (Monacanthidae), which occurred in 34% of samples; the triggerfishes (Balistidae), which occurred in 29%; and the swellfishes and porcupine fishes (Tetraodontidae and Diodontidae), which were found in 35% of the stomachs examined.

Squid occurred in 24% of the stomachs examined and were the only important invertebrate food found.

The balance of the species which comprised the food of the dolphin consisted mostly of small numbers of pelagic fish. Juveniles of the pompano dolphin, *C. equisetis*, and the Serranidae were found frequently. These are usually associated with floating *Sargassum*, as are the Balistidae and Tetraodontidae. The association of *C. hippurus* with floating material is well known by local fishermen (Brown, 1942).

The results from the Barbados specimens collected during the period

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TABLE I. Stomach contents of the dolphin, *Coryphaena hippurus* Linnaeus, and the yellowfin tuna, *Thunnus albacares* (Lowe).

Prey species	Total No. specimens	Per cent occurrence
Coryphaena hippurus (70 specimens)		
Cephalocanthidae — *Dactylopterus volitans* (Linnaeus)	249	56
Exocoetidae – unidentified	78	57
Scombridae – *Auxis thazard* (Lacépède)	2	1
Coryphaenidae – *Coryphaena equisetis* Linnaeus	13	13
Hemirhamphidae – *Hemirhamphus* sp.	9	6
Gempylidae – *Gempylus serpens* Cuvier and Valenciennes	2	2
Serranidae – unidentified	1	1
Synodontidae – *Synodus* sp.	1	1
Carangidae – *Caranx latus* Agassiz	1	1
Nomeidae – *Nomeus gronowi* (Gmelin)	35	2
Balistidae – unidentified	58	29
Monacanthidae – unidentified	133	34
Tetraodontidae – unidentified	47	16
Tetraodontidae – *Lagocephalus lagocephalus* (Linnaeus)	19	8
Diodontidae – unidentified	9	8
Diodontidae – *Diodon hystrix* Linnaeus	2	2
Diodontidae – *Chilomycterus schoepfi* (Walbaum)	1	1
Unidentifiable fishes		36
Cephalopoda – unidentified	24	24
Algae		5
Thunnus albacares (12 specimens)		
Cephalocanthidae – *Dactylopterus volitans* (Linnaeus)	14	25
Exocoetidae – unidentified	1	8
Scombridae – *Auxis thazard* (Lacépède)	6	8
Serranidae – unidentified	1	8
Priacanthidae – *Priacanthus* sp.	19	25
Holocentridae – *Holocentrus* sp.	3	8
Synodontidae – *Synodus* sp.	1	8
Monacanthidae – unidentified	18	33
Monacanthidae – *Amanses* sp.	7	25
Tetraodontidae – *Lagocephalus* sp.	2	16
Ostraciidae – *Lactophrys* sp.	1	8
Unidentifiable fishes		40
Cephalopoda – unidentified	116	91
Crustacea – megalops larvae	13	25
Algae		16

February to June are consistent with those of Erdman (1958) from Puerto Rico and the Bahamas. Erdman (1958) found flyingfishes to be the most important food of the dolphin. He also found that filefishes, triggerfishes, swellfishes, and porcupine fish were important elements in the diet.

The occurrence of the gurnard as an important element in the food of the dolphin in Barbados is an unusual feature. The gurnard specimens were all small juveniles and were not, therefore, as important as flyingfishes in terms of bulk.

Gibbs and Collette (1959) reported fishes to be the most important food items in dolphin stomachs from the Gulf of Mexico and from the Gulf Stream. The Balistidae, Monacanthidae, and Carangidae were the most important families and they noted that fragments of *Sargassum* were found in many stomachs. No flyingfishes were found by these authors but they report that flyingfishes were common in the diet of dolphins at Miami. Ronquillo (1954) reported fish of the families Trichiuridae, Holocentridae, and Carangidae to be most important in stomachs of dolphins in the Philippines.

Although only 12 specimens of *T. albacares* were obtained, it is obvious that the food habits of this species are very different from those of the dolphin (Table I). The most important food species were the cephalopods, most of which were squid. Although some feeding on fish occurred, the small number of flyingfishes in the tuna stomachs was notable.

Erdman (1958) found a high incidence of squid in the stomachs of *T. albacares* from Puerto Rico and from the Bahamas. Thomas (1962) found that small fishes were the most important food of this species, whereas squid were noted as important by Rientjes and King (1953) in the Pacific. Kumaran (1962) found squid constituted 56% of the food volume in stomachs of *Euthynnus affinis*. Squid were found to be important in the diet of tunas by Nakamura (1936), King and Ikehara (1956), and Clemens and Iselin (1962). The food habits of *T. albacares* in Barbados thus appear to be similar to those of tuna elsewhere.

The assistance of the Barbados Game Fishing Club with the collection of specimens is acknowledged. We are grateful to Dr W. B. Scott of the Royal Ontario Museum, Toronto, Ontario, for assistance with the identification of specimens. The study was supported in part by a grant from the Canadian National Sportsmen's Show.

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REFERENCES

BROWN, H. H. 1942. The sea fisheries of Barbados. Development and Welfare in the West Indies, Bull. No. 1. p. 1–32.

CLEMENS, H. B., AND R. A. ISELIN. 1962. Food of Pacific albacore in the California fishery (1955–1961). F.A.O. experience paper No. 30. World Scientific Meeting on the Biology of Tunas and Related Species, La Jolla, California, 2–14 July 1962.

ERDMAN, D. S. 1958. Foods of pelagic fishes from Puerto Rico and the Bahama Islands. Third Intern. Game Fish Conference, Miami Beach, Fla. November 1958. 10 p. (Unpublished.)

GIBBS, R. H., AND B. B. COLLETTE. 1959. On the identification, distribution and biology of the dolphins, *Coryphaena hippurus* and *C. equisetis*. Bull. Marine Sci. Gulf Caribbean, **9**: 117–152.

HALL, D. N. F. 1955. Recent developments in the Barbadian flying-fish fishery and contributions to the biology of the flying-fish, *Hirundichthys affinis* (Günther). Colonial Office, Fish. Publ., **7**: 1–41.

KING, J. E., AND K. IKEHARA. 1956. Comparative study of the food of the big-eye and yellowfin tuna in the Central Pacific. U.S. Fish Wildlife Serv. Fish. Bull., **57**: 61–85.

KUMARAN, M. 1962. Studies on the food of *Euthynnus affinis affinis* (Cantor), *Auxis thzard* (Lacépède), *Auxis thynnoides* Bleeker and *Sarda orientalis* (Temminck and Schlegel). Proc. Symp. Scombroid Fishes, Part II, p. 599–606. Mandapam Camp, S. India.

NAKAMURA, H. 1936. The food habits of the yellowfin tuna, *Neothunnus macropterus* (Schlegel) from the Celebes Sea. Trans. Nat. Hist. Soc. Formosa, **25**: 1–8.

REINTJES, J. W., AND J. E. KING. 1953. Food of the yellowfin tuna in the Central Pacific. U.S. Fish Wildlife Serv. Fish. Bull., **54**: 91–110.

RONQUILLO, I. A. 1954. Food habits of tunas and dolphins based on examination of their stomach contents. Philippine J. Fish., **2**: 71–83.

THOMAS, P. T. 1962. Food of *Katsuwonus pelamis* (Linnaeus) and *Neothunnus macropterus* (Temminck and Schlegel) from Minicoy waters during the season 1960–61. Proc. Symp. Scombroid Fishes, Part II, p. 626–630. Mandapam Camp, S. India.

Bioluminescence in Two Species of Pelagic Amphipods

The earliest published accounts of luminescence in amphipods, by Vivani (1805) and Tilesius (1819), are mostly of historical rather than scientific interest because it is impossible to identify, with any confidence, from their crude representations the amphipods these authors believed to be luminescent. Presumed luminescent organs have been described in the genera *Scypholanceola* and *Streetsia* by Woltereck (1905) and Fage (1934) respectively, but actual luminescence has not been observed in these hyperiids. The luminescence reported in several genera of beach-hoppers (Talitridae) is not self-induced, but is caused by parasitic luminous bacteria (see discussion by Bousfield and Klawe, 1963).

The only observation known to me of self-luminescence in an amphipod is one that I made in 1954, which Harvey (1955, p. 18) included in a table of known luminous animals and mentioned briefly in a later review (1961, p. 171). In the present paper I give a more extended account of this observation and give the details of a second observation of self-luminescence in an amphipod.

The amphipod in the earlier observation was the hyperiid, *Parapronoe crustulum* Claus, a member of the family Pronoidae. The nine specimens collected are deposited in the Division of Crustacea, Smithsonian Institution (Cat. No. 107764). They were dipnetted under a light at night (7:00–7:30 PM) east of Eleuthera Island, Bahamas, January 27, 1954, during cruise 5 of the U.S. Bureau of Commercial Fisheries R/V *Theodore N. Gill*. After being placed in sea water in a small screwtop vial, they were taken to a dark location on the ship. There both Richard B. Williams and I observed repeatedly that vigorous shaking of the vial was followed by a yellowish-green glow over the entire bodies of the amphipods.

The other observation of luminescence in an amphipod was made by Mr Peter McCrery while on cruise 235 of the University of Washington R/V *Brown Bear* in the southern Gulf of Alaska. I am grateful to Dr Karl Banse for calling my attention to McCrery's observations and sending me copies of the two original data sheets on which McCrery recorded his observations. Luminescence by a "pink helmet amphipod" was noted by McCrery at stations 24 (52°50′N, 142°51.5′W, July 21, 1959) and 42 (53°54.5′N, 153°05.5′W, July 24, 1959). At these stations tows were made with a 3-ft midwater trawl at depths of about 60 m (station 24) and 30 m (station 42) for about 30 min. The luminescence, which resulted when formalin was added to the samples, occurred in two areas of the amphipod: an anterior spot "just behind eyes" (station 24) or "on tip of helmet," i.e. the produced 1st pereonite (station 42); and a longer patch on the dorsolateral part of the pleon. The color of the luminescence was an intense greenish- to bluish-white.

Four species of amphipods were collected at station 24 and six species at

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station 42. From McCrery's notes and sketches there is no difficulty in identifying his luminescent "pink helmet amphipod" as *Cyphocaris challengeri* Stebbing (family Lysianassidae), present in the samples from both stations and the most abundant pelagic gammaridean in the North Pacific.

No luminescent organs are apparent in preserved specimens of either *Parapronoe* or *Cyphocaris*, and the sources of their light must await further investigation. It seems likely, however, that integumental glands will be found that discharge a luminous secretion in response to stimulation.

Other species of pelagic amphipods are undoubtedly among the many unidentified animals that contribute to the flashes of light found at all depths in all localities of the oceans so far examined.

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REFERENCES

BOUSFIELD, E. L., AND W. L. KLAWE. 1963. *Orchestoidea gracilis*, a new beach hopper (Amphipoda: Talitridae) from Lower California, Mexico, with remarks on its luminescence. Bull. S. Calif. Acad. Sci., **62**(1): 1–8.

FAGE, LOUIS. 1934. Sur la présence d'organes lumineux chez les amphipodes pélagiques. Compt. Rend., **198**: 1631.

HARVEY, E. NEWTON. 1955. Survey of luminous organisms: problems and prospects. *In* Frank H. Johnson [ed.] The luminescence of biological systems. p. 1–20.

1961. Light production. *In* H. Talbot Waterman [ed.] The physiology of Crustacea. Vol. II. Sense organs, integration & behavior. Academic Press, Inc., New York. Chap. 5. p. 171–190.

TILESIUS, W. G. 1819. Über das nächtliche Leuchten des Meereswassers. Neue Annalen der Wetteranischen Gesellschaft für die gesammte Naturkunde. Vol. 1. Frankfurt am Main, 1819. p. 1–10. Pl. xxi.

VIVIANI, DOMENICO. 1805. Phosphorescentia maris quatuordecim lucescentium animalculorum novis speciebus illustrata. Genuae, 1805.

WOLTERECK, R. 1905. Mitteilungen über Hyperiden der Valdivia-(Nr. 4) der Gauss (Nr. 2) und der Schwedischen Südpolarexpedition. a. *Scypholanceola*, eine neue Hyperiden gattung mit Reflektororganen b. Die *Physosoma*- larve der Lanceoliden. Zool. Anz., **29**(13): 413–417.

A Shrimp Pot for Experimental Fishing

Shrimp of the family Pandalidae are potentially one of Alaska's richest fishery resources. When the U.S. Bureau of Commercial Fisheries began studies of the life histories, ecology, and behavior of these shrimp, shrimp pots were selected as one type of sampling gear. Most of the pots used in commercial shrimp fishing, however, were too heavy and bulky for the research planned. A small, light-weight, circular pot, purchased from a fisherman in Homer, Alaska, was modified, and proved to be effective for experimental use because it captured a large variety of species and a wide range of lengths.

The pots are made with plastic pipe frames, nylon mesh covers, and four tunnel entrances. The materials for each pot cost about $10; a pot can be constructed in 1 hr if the mesh covers are presewn.

A pot with the same basic design but without the tunnel entrances is used for holding live shrimp. These containers are made in a variety of sizes and are excellent for holding shrimp for a year or longer.

Pot Frame. The frame is constructed of ½-inch diam flexible polyethylene plastic pipe (Fig. 1A). The upper and lower hoops are each constructed of four 16-inch pieces of pipe, joined to form a circle with four nylon tees. The two hoops are joined together with four 12-inch pipe uprights. All joints are friction held; no cement or clamps are required. The finished frame is about 22 inches in diameter and 14 inches high. Small holes are drilled in the tees to allow water to fill the hollow frame. When this is done the pot, which weighs 2.2 lb, has slightly negative buoyancy in water. To produce a heavier pot, lead ballast is placed inside the pipe.

Pot Cover. The covering of fine woven nylon mesh has oval openings with a maximum dimension of $\frac{3}{16}$ inch.

Each cover is presewn in two parts — a flat-bottomed, bag-like cover for the bottom and sides of the pot and a flat lid for the top. The cover for the bottom and sides is composed of two pieces of mesh, one a circle the size of the pot bottom (22-inch diam) and the other a rectangular piece as long as the circumference of the frame and somewhat wider than the height of the frame (70 × 17 inches). These are sewn together to form a shallow cylinder with a bottom, and a ½-inch wide nylon elastic band is inserted in a hem around the upper edge. This covering is placed around the frame, and the upper edge is laced to the upper hoop of the frame to hold it firmly in place. The lid is a circle of mesh larger in diameter (approximately 26 inches) than the top of the pot, with a ½-inch wide nylon elastic band inserted in a hem around the edge. The lid fits over the top of the pot and is tied permanently to the upper hoop at three of the tees. This allows the top of the pot to be opened to half of its area without completely removing the lid (Fig. 1B and C.)

Tunnel Entrances. Four tunnel entrances are installed after the pot cover is fastened in place on the frame. Each entrance ring is made of heavy (0.2-

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inch diam) hard-annealed copper wire formed into a circle with a 2½-inch inside diam. The ends of the wire are soldered together to eliminate any gap in the ring. One ring is sewn to the mesh at the center of each of the four sides of the pot (Fig. 1B). After the rings are attached, the mesh within each ring is cut out, and the opposing tunnel rings are pulled together and fastened with twine to form the short tunnels (Fig. 1C).

Other Features of the Pot. A clip is tied to the frame at one of the upper tees (Fig. 1C) to attach the pot to a line for fishing.

Rubber loops cut from tire tubes are fastened to one pair of opposing uprights in the pot frame so that a bait box can be suspended near the center of the pot.

Effectiveness and Durability. The shrimp pots described have been used successfully for research shrimp fishing in Kachemak Bay, Alaska, since 1963. They are generally fished in a long-line arrangement, with several pots attached to a single anchored line. In addition to the usual position on the bottom, these pots can also be fished off bottom because of their light weight. The catch per pot for 24-hr fishing periods is typically over 100 shrimp and has exceeded 600. Most of the shrimp taken are pandalids. The species caught in greatest abundance and the length ranges of their carapaces are:

Species	*Pandalus borealis*	*P. goniurus*	*P. hypsinotus*	*P. platyceros*
Length range (*mm*)	6.0–27.5	5.5–24.0	6.5–40.0	13.0–58.5 .

Shrimp caught in lesser abundance are *Pandalus danae*, *P. stenolepis*, and several species of the family Hippolytidae. A few fish and crabs are also captured.

Since the pots are constructed of noncorroding and nondecaying materials, little maintenance has been necessary. The most frequent repairs have been to torn meshes and to broken or weakened elastic bands in the lid of the pot. With careful handling and a minimal amount of maintenance, these pots can provide an inexpensive and effective method for experimental shrimp fishing.

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Biological Laboratory, Auke Bay, Alaska

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(FIG. 1 *opposite*)

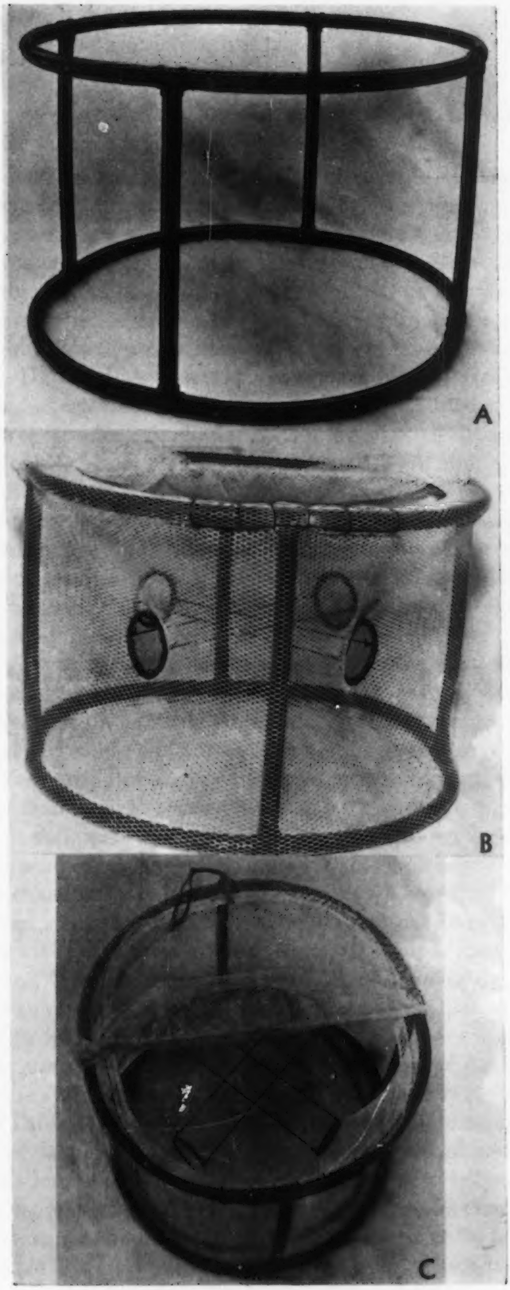

FIG. 1. Three views of shrimp pot used for experimental fishing: (A) assembled plastic pipe frame; (B) side view of pot; (C) top view; note clip attached to frame.

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The Shrinkage Factor in Measurements of Groundfish

The usefulness of information on the size of recaptured tagged fish depends in part on the conditions under which the fish were held in the interval between capture and measurement. Commonly, tagged fish are stored in ice in the hold of a vessel and/or frozen in a cold storage plant ashore. Harry (1956) examined the effects of holding tagged flatfish, of known length, in a study involving three species of the family Pleuronectidae (*Eopsetta jordani*, *Parophrys vetulus*, and *Microstomus pacificus*). The specimens ranged in length from 25 to 60 cm. He concluded that, to compensate for shrinkage resulting from holding on ice, a correction factor of 0.5 cm should be added to the observed lengths for all three species. A similar factor applies to frozen specimens except in the case of *M. pacificus*. For that species 1.0 cm should be added to the observed length.

The correction factor for frozen fish is a useful approximation. However, Harry (1956) noted some indication of proportionality between shrinkage and fish length, which assumes possible importance in species such as *M. pacificus* which has a relatively wide size range. Shrinkage of iced fish, on the other hand, appears, on the basis of available data, to be a constant.

Since annual growth typically decreases with age, the error in uncorrected tagging estimates of growth increments becomes progressively larger with the age of the fish. At a certain age, the correction factor for shrinkage equals or exceeds the annual increment of growth. This is no doubt largely responsible for the common observation of zero or negative growth of tagged fish when in all likelihood there had been positive growth.

The problem of shrinkage also emerges when attempts are made to compare market-length frequencies or age–length data with corresponding data collected from fresh specimens, for instance aboard a research vessel. Differences between fresh and iced samples may emerge when in fact fish of two samples were identical in size in life. Differences may also arise if comparisons are made between whole fish and "frames" (carcasses with fillets removed).

An attempt was made to assess "errors" in length measurements obtained at British Columbia ports from whole and processed carcasses of several species of groundfish. Measurements of three species of flatfish and of Pacific cod (*Gadus macrocephalus*) which had been iced in a vessel hold were recorded before and after processing (Table I). In all species there was an increase in length from the whole to frame length. Amongst the flatfish the percentage increase was greatest in *Eopsetta jordani* (petrale sole) but was, on the average, less than 1% (2.7 mm) for fish lengths of 32–48 cm, with no evidence of trend (in absolute units) within that range. Pacific cod showed an increase in average length from dressed (gutted) to frame length as well as from whole to frame length but the increase again was less than 1% (means 4.5 and 4.8 mm) over the range

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TABLE I. A comparison of whole fish lengths with "frame" lengths as recorded for several species of groundfish stored in ice and landed at the port of Vancouver.

Species	Location of capture	Number of fish sampled	Size range (*mm*)	Avg length (*mm*)		Difference (*mm*)	Per cent increase
				Whole	Frame		
Eopsetta jordani (petrale sole)	Hecate Strait	54	320–480	398.3	401.0	2.7	0.6
Parophrys vetulus (English sole)	Strait of Georgia	105	280–480	363.8	365.7	1.9	0.5
Lepidopsetta bilineata (rock sole)	Hecate Strait	51	310–480	386.7	387.6	0.9	0.2
	Strait of Georgia	105	290–455	361.3	362.2	0.9	0.2
Gadus macrocephalus (Pacific cod)							
Whole length and frame length	West coast of Vancouver Island	88	385–760	588.5	593.3	4.8	0.8
Dressed length and frame length	West coast of Vancouver Island	46	440–760	567.2	571.7	4.5	0.8
Fresh (un-iced) whole length and frame length	West coast of Vancouver Island	55	460–785	598.2	598.7	0.5	<0.1

of sizes observed, 38–76 cm. There was virtually no increase in average length of a sample of un-iced Pacific cod after processing.

In the collection of market samples of groundfish for the Nanaimo station, all flatfish length measurements are based on frames whereas those of Pacific cod are frequently mixed (usually frame or dressed). Measurements are taken to the nearest centimetre.

More by good fortune than design, the practice of taking measurements from flatfish frames appears to compensate in part for shrinkage (when iced) between time of capture and landing.

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REFERENCES

HARRY, G. Y. 1956. Analysis and history of the Oregon otter-trawl fishery. Ph.D. Thesis. University of Washington, 328 p.

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Distribution of Low Mobility Proteins in the Blood of Fishes[1]

In a study of the effects of stress on certain blood parameters, we noted that the amount of protein that remained near or anodal to the application point after paper electrophoresis differed between three species of fish. This suggested that the proportion of the serum of plasma proteins accounted for by this fraction is primarily determined by genetic factors. Therefore, we expanded our sampling to include 22 species of fish and herein report the results of these analyses.

Fish for these determinations were collected from their native habitat or at the hatchery and acclimated to laboratory temperature for several days before their blood was drawn and tested. In a few cases, blood samples were collected in the field as soon as the specimens were captured. Serum was used for most of the analyses, but only plasma was available from some of the species. Presumably, this would affect the results to a minor degree.

The samples were analyzed by the methods and materials described by Bouck and Ball (1965), using Veronal buffer and paper electrophoresis. The fraction of protein that remained at or anodal to the application point (Fig. 1) was expressed in grams per 100 g of sample protein.

The resulting values for the various species were arranged in rank-order, proceeding from the lowest to the highest amounts of this protein (Table I). Salmonids generally had less than half of the average quantity present in Ictalurid serum and yellow perch (*Perca flavescens*) had only a trace of this in their blood. Although these values were obtained from small sample sizes, they occur with regularity and we do not expect additional samples to greatly change the ranks of these species.

Several factors can change the amounts of protein in this fraction including stress (Fujiya, 1961; Bouck and Ball, 1965), different temperature regimes (Meisner and Hickman, 1962), and different methods of capturing the fish (Bouck and Ball, 1966). However, these changes are relatively small and we conclude that the amount of this fraction is primarily controlled by genetic factors.

The amount of low mobility blood protein in the blood of a species is generally associated with what we consider to be a general pollution tolerance. For example, fish that require clean water, such as Salmonids, have very little low mobility protein in their blood. Fishes that survive or even thrive in so-called "polluted" waters have large amounts of low mobility protein in their blood, i.e. bullheads and carp. Fishes that frequent intermediate conditions generally

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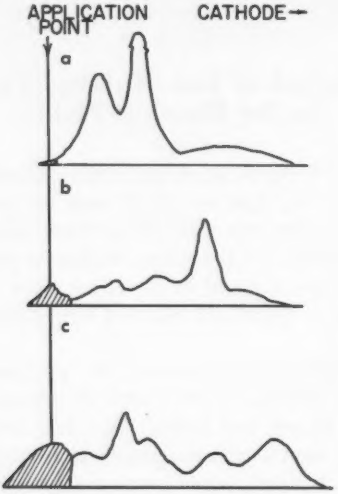

FIG. 1. Electropherograms from fish species ((*a*) yellow perch, *Perca flavescens*; (*b*) rainbow trout, *Salmo gairdneri*; (*c*) yellow bulhead, *Ictalurus natalis*) representing typical low intermediate, and high amounts of the blood protein fractions of interest (shaded areas). Paper electrophoresis was conducted using Veronal buffer at *p*H 8.6.

TABLE I. Amounts of low mobility protein in the blood of 22 species of freshwater fish.

Rank	Species	No. of specimens		Average grams of protein per 100 g of sample protein
1	Yellow perch (*Perca flavescens*)	9	Plasma	Trace
2	Smallmouth bass (*Micropterus dolomieui*)	3	Serum	2.7
3	Grass pickerel (*Esox americanus vermiculatus*)	5	Serum	3.1
4	Brown trout (*Salmo trutta*)	4	Serum	3.5
5	Brook trout (*Salvelinus fontinalis*)	4	Serum	4.7
6	Rainbow trout (*Salmo gairdneri*)	5	Plasma	5.4
7	Black crappie (*Pomoxis nigromaculatus*)	2	Serum	5.9
8	Northern pike (*Esox lucius*)	6	Serum	7.5
9	Largemouth bass (*Micropterus salmoides*)	9	Serum	7.8
10	Pumpkinseed (*Lepomis gibbosus*)	1	Serum	7.9
11	Rock bass (*Ambloplites rupestris*)	6	Plasma	8.8
12	Bluegill (*Lepomis macrochirus*)	10	Serum	8.8
13	Goldfish (*Carassius auratus*)	10	Serum	9.1
14	Black bullhead (*Ictalurus melas*)	2	Plasma	10.3
15	Lake chubsucker (*Erimyzon sucetta*)	1	Serum	12.2
16	Bowfin (*Amia calva*)	3	Serum	12.3
17	Hogsucker (*Hypentelium nigricans*)	2	Plasma	13.1
18	Channel catfish (*Ictalurus punctatus*)	1	Serum	14.5
19	Green sunfish (*Lepomis cyanellus*)	2	Serum	17.9
20	Yellow bullhead (*Ictalurus natalis*)	7	Serum	18.3
21	Carp (*Cyprinus carpio*)	2	Plasma	20.2
22	Brown bullhead (*Ictalurus nebulosus*)	1	Serum	21.5

have intermediate amounts, i.e. Centrarchids. In the light of this, it is important to point out that these fish were held in clean water for several days before being tested.

The biological significance of this protein fraction in fish blood is not completely resolved at this time. Its mobility is similar to that of gamma globulin, but a similar mobility does not necessarily denote similar physiological or biochemical function. However, antibodies have been found in the low mobility protein fraction of *Salmo gairdneri* by Post (1963) and in *Notemigonus crysoleucas* by Summerfelt (1966). In *S. gairdneri*, this fraction also contained fibrinogen and other enzymes (Bouck and Ball, unpublished data). Undoubtedly, the fraction is a highly complex mixture of proteins that are close to their iso-electric point at *p*H 8.6 in Veronal buffer, hence, probably have more reactive sites than the more mobile proteins.

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REFERENCES

Bouck, G. R , and R. C. Ball. 1965. Influence of a diurnal oxygen pulse on fish serum proteins. Trans. Am. Fish. Soc., **94**: 363–370.

1966. Influence of capture methods on blood parameters and mortality in rainbow trout (*Salmo gairdneri*). Ibid., **95**: 170–176.

Fujiya, A. 1961. Use of electrophoretic serum separations in fish studies. J. Water Pollution Control Federation, **33**: 250–257.

Meisner, H. M., and C. P. Hickman, Jr. 1962. Effect of temperature and photoperiod on the serum proteins of the rainbow trout *Salmo gairdneri*. Canadian J. Zool., **40**: 127–130.

Post, G. 1963. The immune response of rainbow trout (*Salmo gairdneri*) to *Aeromonas hydrophila*. Dept. Inform. Bull. 63-7, Utah Dept. Fish Game. 82 p.

Summerfelt, R. C. 1966. Identification of a fish serum protein with antibody activity. Bull. Wildlife Disease Assoc., **2**: 25–30.

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Guide to Contributors

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